

nature

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There's more to telecommunications . . .

THE Science Research Council (SRC) has recently come out with a report by one of its specialist subject panels which deserves a wide readership, both because it raises some fundamental issues about scientific research in universities and because the topic with which it deals—telecommunications—plays such an intimate part in everyday life. The report considers the SRC's own support for telecommunications research in universities and polytechnics, and does some plain speaking about the nature of the research itself and the environment in which it is carried out (*Telecommunications*, Science Research Council, State House, High Holborn, London WC1R 4TA).

First of all, the panel contends that the emphasis in university telecommunications research is in the wrong place. Basically long-term work on, for example, signal processing and systems studies is eschewed, the panel says, in favour of transmission and propagation studies: as a rough guide 15 out of 31 SRC research grants current in June 1974 were for work on transmission lines, waveguides, antennas and propagation. On the other hand only three grants were awarded for studies of communications networks.

The drift of the panel's recommendations, which the SRC is putting about for discussion and comment, is not hard to guess. It wants the SRC to make a gradual transfer of funds to the research topics it highlights as 'special areas'. It also thinks that "existing university effort [in telecommunications research], with one or two exceptions, is too thinly distributed to be effective for the broader inter-disciplinary research required . . ." In other words, the SRC is being asked to implement its formula of selectivity and concentration.

The panel adds a rider, however, to the effect that there should still be funds available for "individual workers" to research on "limited aspects" of telecommunications. It says, however, that it does not want to see unnecessary duplication of work done, for example, at its own Appleton Laboratory (formerly the Radio and Space Research Station).

As far as research in universities in general is concerned there are two main questions that arise—whether the universities themselves should be thinking more along the lines of selectivity and concentration, unprompted by bodies like the SRC, in the light of the present stagnation of university funds, and whether postgraduate students would be better off if the doctrine of selectivity and concentration were practised more widely. If more universities are ever again set up in Britain one hopes desperately that they will not feel it

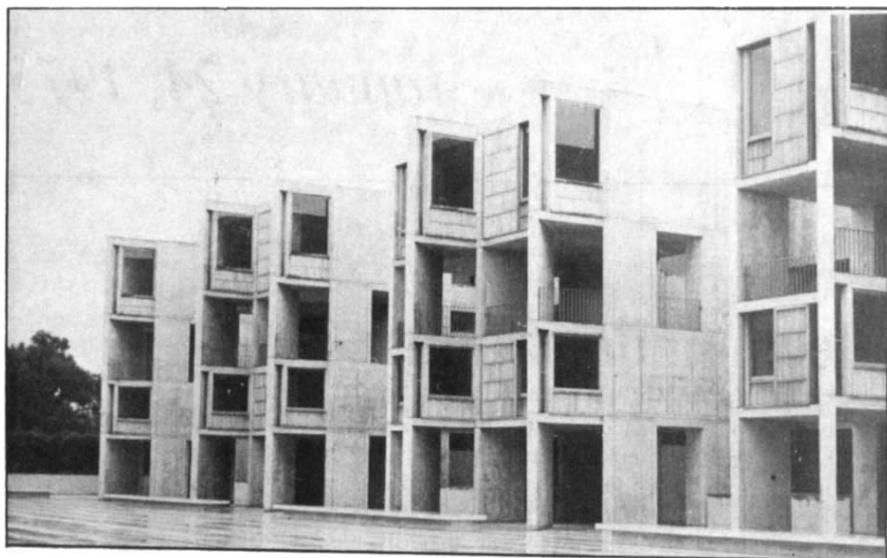
incumbent upon themselves to have, say, a geology department just because every university has to have one. There seems little doubt that the research student is better off in the long run in a department that is both large and broadly based—so that he emerges not as a diluted specialist but as a good specialist with a generalist aura about him. Interestingly, the report shows industry apparently facing in several directions over the usefulness of a PhD in telecommunications—some companies are adamant that the PhD training is irrelevant, whereas one says that "the best young members of its research staff have almost all received PhD training". Both views, however, probably crystallise the underlying frustration within companies that many good graduates do PhD courses before entering industry, and imply that it would be nice if that training created more advantages to the companies concerned. The SRC panel seems to deal sensitively with this kind of thinking.

Leaving university research aside, the panel's report is also important in that it spells out a catalogue of research that needs to be done in what has become an all-pervading aspect of modern life. At a time when broadcasting in Britain seems to be going through a rough patch, academics could well get their teeth into an examination of the pros and cons of broadcasting and cable systems for television, or take a fundamental look at the most effective way of using the frequency spectrum for radio, to name two of the panel's own examples. Then there are the possibilities opened up by the idea of integrated local telecommunications services—carrying telephone signals, for example, along with radio, television and other signals—and the desirability of refining conventional telephone systems to accommodate the special needs of groups like the old and the disabled.

The panel might well also have included a mention of the links between good telecommunications and the conservation of energy and other resources, though this is implicit in the idea of integrated local services. Even now a telephone conversation is often quicker and cheaper in energy than writing a letter, and projects like CEEFAX (which would provide instantly accessible news and other information on a domestic television set) are likely to have an impact on the present energy-intensive means of disseminating information through newspapers.

The SRC plainly thinks the universities have much to offer, and it can now only sit back and see if the appropriate response is forthcoming. □





Salk of human kindness

Armed with promises of financial support, 27 acres of prime building land donated by the citizens of San Diego and a strong conviction that science should be used for the good of man, Jonas Salk, an immunologist whose name became a household word when he developed the world's first polio vaccine, began in the late 1950s to plan a new venture. He wanted to establish a biological research centre dedicated "to contributing . . . to the health and well-being of man". The resulting Salk Institute for Biological Studies, now 11 years old, is a remarkable place. Colin Norman reports.

SET atop low cliffs overlooking the Pacific Ocean, just up the coast from the Scripps Institution of Oceanography and a stone's throw from the San Diego campus of the University of California, the institute probably ranks among the best known scientific establishments in the world. Salk's name alone is enough to ensure a good deal of publicity for the place, but it has also been pushed into the limelight through the works of one of its fellows, the late Jacob Bronowski, whose epic television series "The Ascent of Man" opened in North America this month. And, within the scientific community, the institute has already carved out a considerable reputation for itself in several areas of biomedical science.

Equally celebrated is the institute's stunning architecture. A striking concrete and glass design endowed with more than a touch of cubism, the structure is in fact so well known that the place has become something of a tourist attraction and the buildings have even been used on several occasions as a backdrop for fashion photographs. (Permission to film a battle scene in the central courtyard was, however, denied on the grounds that it would have been rather distracting for the scientists.) As well as attracting tourists and photographers, the buildings have also spawned reams of prose

in publications ranging from textbooks on modern architecture to *Time* magazine, the prize for which must surely go to the following florid description: "A spread of related buildings (conceived) to propitiate the gods of science even as the ancient Athenians built the Acropolis to keep their gods happy".

But how does the institute propitiate the gods of science in a manner different from other, less glamorous, research establishments? Salk's intention was to create an institute where research would be devoted primarily to health problems, but he also wanted to add an extra dimension of humanism to the place to ensure that the research is indeed, devoted to enhancing the well-being of man—biology with a conscience, Salk has called it.

In several respects, the institute has turned out much as its founder intended, but the grand design has altered a little over the years—"evolved is a better word", Salk insists—and there have recently been some significant changes in the way in which the institute operates. It has also run into its fair share of problems, not the least of which have been a chronic shortage of funds and, until recently, some discontentment among the younger researchers there.

Salk says that he first conceived the idea of founding a scientific institute in 1956, two years after his momentous

development of a killed polio virus vaccine. He was assisted in the planning by a number of prominent scientists, among whom the nuclear physicist Leo Szilard is said to have been influential, and his chief supporter was Basil O'Connor, the President of the National Foundation-March of Dimes (NFMD). The project began to take concrete shape in the early 1960s with the help of the architect Louis I. Kahn and financial backing from the NFMD (which also supported Salk's work on the polio vaccine), on a plot of land in the wealthy San Diego suburb of La Jolla. The first senior scientists were appointed in 1963.

The basic idea was to bring together a number of eminent scientists and give them tenure, freedom from teaching duties, a large amount of laboratory space, and it was hoped an adequate budget for their work. These so-called resident Fellows would provide overall scientific direction for the institute themselves, with the outside help of a panel of equally eminent non-resident Fellows who would meet once a year, advise on major new projects and on senior staff appointments.

The first resident Fellows reflected in large measure Salk's background in immunology and virology. Appointed in 1963 were the immunologists Melvin Cohn and Edwin Lennox, both of whom joined Salk from the Pasteur Institute, the virologist Renato Dulbecco, who moved down the coast from the California Institute of Technology (and who has since left to join the Imperial Cancer Research Fund in London, although he is still technically a resident Fellow of the Salk) and Jacob Bronowski, the mathematician, philosopher, broadcaster and humanist, who joined the Salk Institute from the British National Coal Board.

The next fellow appointed was Leslie Orgel who moved to the institute from the University of Cambridge in 1965 to continue his research into the chemical evolution of life. The Nobel Prizewinner Robert Holley came to La Jolla from Columbia University in 1968, and has since been working on the factors that control cell growth and division. And the latest senior appointment was Roger Guillemin, who moved from the Baylor College of Medicine in Houston to the Salk Institute in 1970, where he has continued his research into the control of the pituitary—work which recently led to the discovery of a new hormone produced in the hypothalamus, called somatostatin, which may be useful in the treatment of juvenile diabetes.

A common theme among those eight resident Fellows is that most of them have broad backgrounds—a factor which Salk says was deliberately sought when the appointments were being con-

sidered. Lennox, for example, was a nuclear physicist before he turned to immunology, Holley worked on nitrogen fixation and transfer RNA before he became interested in cell division, Orgel moved into prebiotic synthesis from inorganic chemistry, Bronowski started out as a mathematician, applied his skills to problems of evolution, developed a smokeless fuel, and was active in writing and broadcasting, and Salk has lately been engaged in writing on philosophy and ethics.

The non-resident Fellows, who meet in La Jolla in January each year to discuss the scientific affairs of the institute, include Nobel Prizewinners Jacques Monod, Gerald Edelman and Salvador Luria, and the President of MIT, Jerome Weisner. Although they play less of a role in the institute's affairs than they did during its early days, their input is still significant. Luria, for example, says that he often feels "more involved in decisions at the Salk than at MIT".

Until relatively recently, the scientific work of the Salk Institute was almost totally dominated by the research interests of its eight resident Fellows, each of whom heads a research group. In the early days, for example, much of the grant money flowing into the institute was channelled through the Fellows to members of their research group—a situation which led to some disenchantment on the part of younger scientists who wanted to pursue their own lines of research. Another cause of frustration was the fact that only the Fellows had tenured appointments; the other scientists were, in theory, less secure at the Salk than their colleagues were in the universities.

But a number of factors have recently altered both of those causes of disharmony, so that the Salk Institute now seems a relatively harmonious place to work in. Perhaps the most significant development has been the setting up of a number of independent research groups consisting mostly of younger scientists working in such fields as neurobiology, cancer, linguistics and reproductive biology. Their work is not tied to that of any of the Fellows, and according to one long-standing member of the institute, the result has been a great improvement in communications between the major research groups there. Another scientist put it more vividly: "a few years ago, nobody talked to anybody else; now the communication is excellent". One particular area which has recently benefited from such collaboration is that of cell division, where studies in three different laboratories at the Salk have recently converged to provide some clues to the factors which cause cells to grow and divide.

As for the question of tenure, last

year eight members of the institute were given the title of Associate Research Professor, and given conditions of tenure similar to those which pertain in the universities. For some of the younger scientists at the Salk, the fact that there is now demonstrably a career structure in the place could be an important factor in persuading them to stay there.

But the institute still has its problems. Like most centres of learning these days, it is short of funds, with the result that it has been difficult for the institute to move into major new areas of research. Another consequence is that the institute's work is now heavily concentrated in cancer research, largely because cancer money is relatively easy to come by compared with funds for other areas of science. A little over half of the institute's work is now concerned with cancer and related studies.

Salk points out, however, that since the institute is interested in "whatever seems to be in the forefront of advancing knowledge", cancer research is a natural area to be involved with, and it is also a good area for attracting bright young scientists.

The institute has also been forced recently to live much more off government funds than it used to. Last year, for example, some \$4.5 million of the \$7 million operating budget came from government grants and contracts. The rest came roughly in the form of a \$1 million grant from the National Foundation-March of Dimes, about \$1 million from other foundations, and \$0.5 million from private donations.

The financial affairs of the institute have, however, recently been placed on a firmer footing by the appointment of Frederic de Hoffmann as President and chief administrator. A nuclear physicist who worked on the Manhattan Project, de Hoffmann has, according to one Fellow of the institute, "brought a sense of financial responsibility" to the

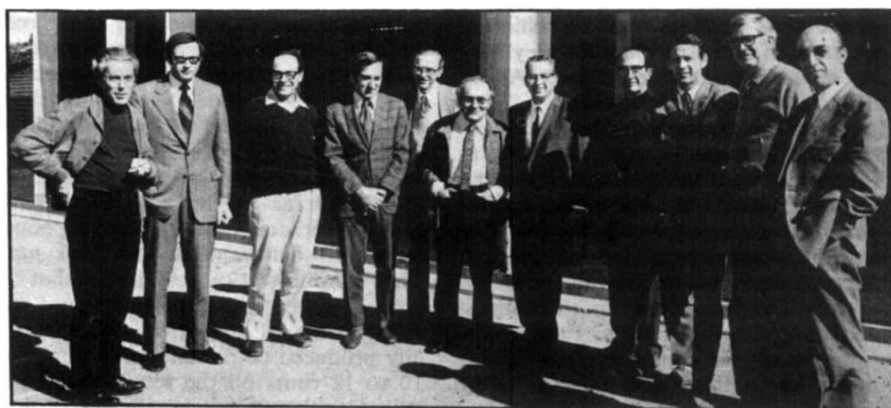
place, and he is also credited with providing the impetus for many of the recent changes such as the establishment of more independent research groups and the extending of tenure to scientists below the rank of Fellow.

One measure of the increased financial stability is that the institute is hoping soon to raise sufficient money to begin a major new programme in neurobiology. Because plans for several large programmes had to be shelved when funding became tight in the early 1970s, some 35% of the laboratory space in the institute has never been occupied, and so the neurobiology programme, if it gets under way, will still leave the institute well short of its maximum capacity.

Another area in which there has been talk of expansion is the institute's human affairs programme. But that received a severe setback last August by the death from a heart attack of Jacob Bronowski, and it is yet to be determined how that side of the institute's activities will develop. Although Bronowski's work did not impinge much on the activities of most of the other scientists in the Salk Institute his range of interests clearly added an extra dimension to the place—a dimension which Salk says "will continue so long as I am here". A scientist closely connected with the institute suggested, however, that it has "never really managed to be a place where biology and the humanities were fused", chiefly because Bronowski's human affairs programme involved too few people. "A critical mass was never reached", he suggested, and there were consequently too few areas of overlap with the rest of the institute's members.

Be that as it may, the Salk Institute now seems to have entered a period of slow growth, paced chiefly by the availability of funds. After its first eleven years of evolution, it remains, in Luria's words, "overall, an exciting place". □

Some of the resident and non-resident Fellows of the Salk Institute. From left to right: Jacques Monod, Gerald Edelman, Melvin Cohn, Leslie Orgel, Robert Holley, the late Jacob Bronowski, Frederic de Hoffmann, Salvador Luria, Paul Berg, Edwin Lennox and Roger Guillemin.



international news

THERE used to be a standing joke in Washington that whenever Mr Nixon wanted to demonstrate that his besieged Administration was in control of events, he would announce a set of new policies for dealing with the energy crisis which were mostly so ineffectual that he usually succeeded in demonstrating just the reverse.

Although the energy policy announced last week by President Ford has run into many of the criticisms that greeted Nixon's plans, and although it stands virtually no chance of being approved by Congress, it at least takes a tougher stand on some of the more controversial issues. And it will also receive much more serious consideration than some of the proposals that have gone before.

Ford's plans, announced as part of the State of the Union message, fall into three parts. During the next three years, he wants to cut 2 million barrels of oil a day from imports, chiefly by employing a few moderate conservation measures, allowing energy prices to increase, and relaxing some environmental controls on the burning of coal. For the medium term, he has proposed a number of measures to increase domestic energy supplies in order to make the United States "invulnerable to economic disruption by foreign suppliers by 1985". In the longer term, Ford reckons that the United States can "develop [its] energy technology and resources so that [it] has the ability to supply a significant share of the energy needs of the free world by the end of this century".

Few people have quarrelled with the goals, but many have taken exception to the means. In particular, Ford's conservation and environmental proposals have drawn heavy sniping from Congress, and his plans for stepping up domestic energy supplies have met with doubts that they can be achieved, mixed with criticisms because they may be environmentally destructive. All in all, the chances that they will be approved on Capitol Hill look slim.

As far as the short term proposals are concerned, Ford has announced that he will put a tariff of \$3 a barrel on imported crude oil by April this year. He has the authority to do that himself, without the approval of Congress, and so unless Congress acts to stop him it will be done. In addition, Ford wants Congress to pass legislation to allow the prices of natural gas and domestic petroleum to increase, and he has asked

Ford spells out his energy plans

by Colin Norman, Washington



Ford: three-point plan

Congress to amend the Clean Air Act to allow power stations in polluted areas to switch from oil and gas to coal. Finally, to prevent oil companies from making excessive profits from the energy crisis—as they have been doing for the past two years—Ford wants Congress to agree to legislation which will put a heavy tax on profits not ploughed back into research and development or exploration.

The need for some swift action is obvious. Although energy consumption decreased during and immediately after the Arab oil embargo, it has recently begun to increase so that the United States is now importing about 7.3 million barrels of oil a day, compared with 6.5 million barrels immediately before the embargo. If no conservation measures are taken oil imports are predicted to increase to about 8 million barrels a day by the end of 1977.

But will Ford's proposals do the trick? As far as the consumption of gasoline is concerned, there is good reason to believe that they will not. The Administration estimates that a combination of the import duty and the deregulation of the price of domestically produced petroleum will add about 10 to 12 cents on the retail price of a gallon of gasoline. In the first nine

months of 1974, however, gasoline prices jumped by about 40% in some parts of the country but consumption has been increasing during the past three months by about 300,000 gallons a day. (It did, however, drop by some 3.4% early in 1974, although some of that may have been because people had to queue for hours to get any gasoline at all.

Nevertheless, the Administration predicts that the higher prices will decrease imports of oil by 1 million barrels per day in 1975 and 1.6 million in 1977, while conversion of oil-fired power stations to coal will save about 300,000 barrels a day of oil imports by 1977.

For the medium term, Ford has quietly dropped his predecessor's oft-proclaimed goal of energy independence by 1980, and he has adopted instead the much more realistic aim of reducing oil imports to between 3 million and 5 million barrels a day by 1985—a level which, he says, will preclude economic disruption from a sudden shutoff of foreign supplies. To achieve such a goal, he has proposed a programme to bring on line within the next 10 years 200 nuclear power plants, 250 major coal mines, 150 major coal-fired power plants, 30 new oil refineries, 20 plants to produce 'synthetic' fuel from coal gasification and liquefaction and from oil shale, "many thousand" oil wells, and the insulation of 18 million homes.

All that will require Congress to approve legislation which will expedite the licensing of new nuclear power plants, allow strip mining of large amounts of coal from the western United States, provide tax credits for insulation of houses, allow the leasing of tracts of land on the outer continental shelf for oil drilling, and extend the time period in which power plants must remove sulphur emissions. In addition, Ford has asked Congress to approve an amendment to the Clean Air Act which will freeze controls on automobile exhaust emissions at essentially the levels to be imposed on 1976 car models, and he has also asked for authority to purchase facilities for a national oil storage depot capable of holding 1,300 million barrels of crude oil.

That package of proposals is likely, however, to draw opposition from Congress on a number of counts, chief of which is the inherent retreat from a number of painfully conceived environmental goals. Consider, for example,

the proposal to delay automobile emission standards. Ford has proposed that strict emission controls which were to come into effect in 1976 should be put off until 1981, in exchange for which the automobile manufacturers have promised to improve by 40% the average gasoline consumption of new American cars by 1980. But Senator Edmund S. Muskie, who heads a key subcommittee which will consider the proposals, has already denounced it because it "trades public health for fuel economy".

Muskie and a number of other observers have pointed out that fuel consumption of 1975 car models was nearly 14% better than that of 1974 models, in spite of the fact that Detroit had to meet strict pollution control standards for the later models. They therefore point out that automobile manufacturers can meet the fuel economy goal without sacrificing environmental controls. Furthermore, a committee of the National Academy of Sciences reported last year that it could see no technical reason for relaxing the standards, and that the cost of meeting them is outweighed by the benefits.

The plans for expediting the nuclear licensing programme may also draw fire from Congress because there is a small but growing number of nuclear sceptics on Capitol Hill, and the Joint

Committee on Atomic Energy, which would normally shepherd such a bill through the Congress, has been considerably weakened by the retirement and defeat of nearly a third of its members. To help head off opposition to the nuclear programme, however, Ford has proposed increasing the budget for research on nuclear safety, waste management and safeguards by \$41 million next year.

A more fundamental question raised by Ford's proposals is whether the United States has the capacity to achieve even the redefined goal of energy independence. In the past few years, according to the Administration's own figures, electricity utility companies have scrapped or postponed 60% of their plans to build nuclear power plants and 30% of those for non-nuclear plants, and this at a time when Nixon was urging all-out expansion to meet the 1980 independence deadline.

The reason was lack of capital for investment, and so Ford last week proposed a number of measures to attract investment capital to the utilities, and to allow electricity rates to increase steeply. That is a prospect which many legislators will not relish explaining to their constituents when they come up for re-election in 1976.

Furthermore, a committee of the National Academy of Engineering pro-

duced a study last year which simply tallied all the measures that would have to be taken to meet the goal of zero oil imports by 1985, the total of which was so staggering that the committee concluded that it is highly unlikely that the goal could be achieved. Although the redefined goal will be easier to meet, there is nevertheless considerable doubt that the capacity can be established in time.

For the long term, Ford has reiterated the Administration's commitment to energy research and development, and has indicated that nuclear power is expected to play a central role in the energy mix towards the end of the century, by which time he hopes that the United States will be so flush with energy that it can export some to the rest of the world. Although he was not very specific about which technologies will get preferential treatment, a background statement distributed with Ford's message at least indicates that the breeder reactor may have slipped a little in the list of priorities. It used to be the number one energy programme in the Nixon Administration, and although the statement indicates that some means must be found to eke out uranium resources, it says that "the breeder reactor is only one such supply source" under consideration. □

THE Administration's plans for promoting the development of nuclear power in the United States were sharply rapped from both sides last week. Two groups of scientists, each replete with Nobel Prizewinners, traded statements in Washington urging, on the one hand, a greater commitment to nuclear power for meeting energy demands and, on the other, criticising the Administration for putting too many of its eggs in one basket.

Although such sentiments are far from new, seldom have they been placed in starker contrast.

The pro-nuclear statement, which was largely the work of the physicist Hans Bethe, was released at a press conference the day after Ford unveiled his energy plans. Signed by 34 eminent scientists, including eleven Nobel Prizewinners, the statement began: "We, as scientists and citizens of the United States, believe that the Republic is in the most serious situation since World War II", and it goes on to deplore the fact that long range energy plans are emerging too slowly.

The statement urged much greater commitment to the use of coal and nuclear power. "We can see no reasonable alternative to an increased use of nuclear power to satisfy our energy needs", it states, while "coal is irreplaceable as the basis of new synthetic

fuels to replace oil and natural gas". The statement also insists that although conservation is desirable, large cuts in consumption can be made only at the expense of jobs.

Asked why he drafted the statement, Bethe said that he had "felt for some years that nuclear energy was not getting enough emphasis", although he said that Ford's energy proposals—which were announced after the statement was written—are a great improvement. Among those who endorsed the statement were William O. Baker, President of Bell Laboratories, Harold Brown, President of CalTech, Joshua Lederberg of Stanford University, Franklin Long of Cornell University, Edward Purcell of Harvard University, Glenn Seaborg, former chairman of the US Atomic Energy Commission (AEC), Frederick Seitz, President of Rockefeller University, Edward Teller of Lawrence Livermore Laboratory, and Richard Wilson of Harvard University.

But critics of nuclear power have also taken exception to the Administration's nuclear plans, though from a different standpoint. On the day that Bethe released his statement, Ralph Nader sent a letter to President Ford urging him to "personally review the implications of dependence on nuclear power", citing "the unique and substantial hazards associated with the

massive amounts of radioactive materials that would inevitably be created by a full scale nuclear power program". The letter was endorsed by eight eminent scientists.

Nader's letter notes that nuclear accidents could endanger "our children and their children, for generations" and states that early enthusiasm for nuclear power has "been steadily eroded as the problems of catastrophic accidents, long-term waste disposal, and the specific hazards of plutonium have been more fully appreciated". In contrast to which, Bethe's statement says that nuclear critics "lack perspective as to the feasibility of non-nuclear power sources and the gravity of the fuel crisis", and expresses "confidence that technical ingenuity and care in operation can continue to improve the safety in all phases of the nuclear power program".

Although neither statement will have much impact on the Administration's plans, they do at least testify to the wide divergence of opinion within the scientific community on nuclear safety. And a heated exchange at the press conference between the chairman, Ralph Lapp, and Daniel Ford, a spokesman for a group which has played a leading role in opposition to nuclear power, confirmed that those opinions are very firmly held.

WORD is getting about that next year's federal grants for research in Canada will be greater than they have been for some years. How much greater no one will say until the official announcement is made (possibly in February), but they will probably increase at least a little in real terms.

Since 1975 will be the year of the new federal granting structure, this may do something to restore the flagging faith of Canadian scientists in their government's interest in science—flagging chiefly because of the steady decrease in research funds available relative to costs and inflation.

At the same time, student enrolment has risen steeply. In six years, starting with 1968, the total Canadian student population increased by about 30%. But in the same period, biology enrolments alone, for example, increased almost 130%. And in eastern Canadian universities the increases were even greater—45% for total enrolment and 190% for biology. During the same period there was an increase of 70% in the number of full-time biology teachers.

But neither operating budgets nor research grants have kept pace. One chairman of a biology department reported a doubled student enrolment in five years coupled with a decline of 20% in his operating budget.

Since 1969-70, the total increase in parliamentary appropriations to the National Research Council (NRC) of Canada for research grants and graduate scholarships has been only 7%. Yet during the same period the cost of research is estimated to have risen by about 50%, and inflation in the cost of scientific equipment and materials has been even greater.

Thus the effective investment in research covered by the NRC grants has actually been reduced by more than a third. The Medical Research Council did little better.

Pronouncements by the government that reorganisation of the granting councils is going to take place this year have done little to reassure the scientists. Last February's Speech from the Throne, in which the announcements were made, "indicates only a concern for procedure and administration", said Dr J. A. Morrison, Director of McMaster University's Institute for Materials Research. "If the present trends continue, there may not be any academic research to administer within five years."

Now it looks as though the government intends to modify its policy somewhat. Late last year, the government approved a supplementary grant of \$2.5 million for the Medical Research Council. And in a House of Commons session last November, C. M. Drury, Minister of State for Science and Tech-

Canadian government ponders MOSST's mission

from David Spurgeon, Ottawa

nology and former Treasury Board President, indicated that the situation was being studied sympathetically. Given the increase in prices and inflation, he acknowledged, "it would be necessary to raise the amounts [of grants] for university research."

Another minister, Hugh Faulkner, the Secretary of State whose department is responsible for grants for the social sciences and the humanities, tried to reassure academics on the reorganisation of the granting bodies in a speech to the Association of Universities and Colleges of Canada; this is another matter that has been vexing them recently.

"No radical changes in the granting policies and practices are sought," he said. "... I can assure you that in drafting the bill, which will be tabled in the course of this session of Parliament [probably not before April or May] every possible effort is being made to take into account the fundamental concerns of the academic community."

The most effective guarantee of the acceptability and effectiveness of these councils will lie in the quality of those appointed to them, the minister said, and "the government intends to give its closest attention to this matter before making its final decisions."

Such reassurances were needed because, altogether, 1974 was not a very good year for instilling in scientists confidence in the government's approach to science policy. Part of the problem was that everybody was trying to figure out what the new Ministry of State for Science and Technology (MOSST) was up to. Conceived in controversy and born in discord (in 1971) the MOSST seemed to be suffering from an identity crisis.

The case was admirably—if rather

Drury: last-ditch effort.



critically—set out in a report published by another government creation, the Science Council of Canada, which has the independence to hire consultants, who do studies for it which it then publishes. This one was called *Knowledge, Power and Public Policy* (Science Council of Canada Background Study No. 31, Information Canada, Ottawa). Its authors were Peter Aucoin, a political scientist, and Richard D. French, a science historian.

The study examined the concept of the ministries of state, which were established with the authority of a 1970 bill to be responsible for designated policy fields not encompassed within the jurisdiction of any single existing government portfolio. The two such ministries studied were science and technology, and urban affairs.

"The ministers of state", say Aucoin and French, "would be faced with a novel task. The organisations that would serve them would not be departments in any traditional sense, but rather ministries whose initiatives would inevitably and consistently involve the responsibilities of other ministries. Fundamental to the notion of a ministry of state is the idea that the activities of research and policy analysis can provide an adequate basis for successful policy formulation and co-ordination. The logic underlying such a ministry derives from the 'knowledge-is-power' hypothesis: namely, that research, information and analysis will carry the day in Cabinet and Cabinet committees against the traditional sources of political and bureaucratic power."

They do not see the concept as a great success so far. Concerning the two ministries studied, they say: "Neither Ministry of State can be said to have had the kind of policy success that was envisaged when they were created." And concerning the MOSST: "The performance of the ministry in relation to the scientific and technological community can hardly be considered a success to date [early 1974]."

Aucoin and French conclude that "the most promising strategy for the MOSST may well be a more modest, more pragmatic, more incrementalist, and less visible role than heretofore", one with a non-threatening service posture rather than a directive one.

There are signs that the suggestion is being heeded. Some see Mr Drury and his deputy as having been sent to make a last-ditch effort to clear up the ministry's problems, in an attempt to determine whether it really can survive or not. And Drury is reported to have commented privately, "If you want to know what will happen to the MOSST, read the Aucoin-French report and use your common-sense." □

THE next move has now been made in the discussions on the desirability and safety of certain types of experiment aimed at producing microorganisms containing new combinations of genetic material. According to the Working Party set up last August by the Advisory Board for the Research Councils under the chairmanship of Lord Ashby, the potential hazards inherent in many of the new techniques for manipulating the genetic make-up of bacteria can be sufficiently minimised to allow the work to continue.

The problem was brought to the attention of the public by a group of American scientists who had pioneered these techniques. A committee of the National Academy of Science under the chairmanship of Professor Paul Berg proposed a moratorium on several lines of research: the construction of new plasmids containing combinations of virulence or drug resistance genes not found naturally, and the transfer of such plasmids into organisms in which they do not already occur naturally; and the linkage of DNA from tumour viruses to plasmids or other viral DNA. Much of the danger from these experiments stems from the fact that the bacterium most commonly used as host is the common gut bacterium *Escherichia coli*, which might be the means of disseminating the new genetic combinations amongst humans with unpredictable results.

Conventionally trained microbiologists and bacteriologists have often regarded with some horror the cavalier way in which molecular biologists and biochemists have tended to treat the organisms with which they work, and can perhaps be forgiven for regarding the current concern over bacteriological hazards with some sense of *déjà vu*. Their answer to the problems posed by possible novel organisms is that with proper precautions these bacteria can be contained in the same way that dangerous pathogens have been successfully contained in the past.

While recognising that the cases are not exactly parallel, the Working Party in its report comes down firmly in favour of the view that proper precautions can indeed reduce the risks from experiments in genetic manipulation to an acceptable level. They recommend that all those working with the new techniques should be trained in dealing with pathogenic bacteria and should have access to expert advice on the precautions necessary in any given case. All laboratories contemplating such work should be properly equipped and very hazardous experiments should only be carried out in special laboratories.

Certain simple and relatively inexpen-

sive precautions, such as no smoking, eating or drinking in the laboratory, wearing gowns and gloves which are removed before leaving and the sterilisation before disposal of all contaminated material, should suffice in most cases. More sophisticated procedures are available for dealing with material such as tumour viruses where the risks are potentially greater. There are also ingenious ways in which bacteria can be to some extent "disarmed" by muta-

Round Britain



tions which do not allow them to grow at above a certain temperature or without some rare growth factor.

Other recommendations include the epidemiological monitoring of all those working with the new techniques, special precautions to be taken when dealing with large-scale experiments and investigation into systems which might prove less hazardous than the commonly used *E. coli*-drug resistant plasmid combination.

The report should provide a timely basis for discussion at the conference called by Professor Berg and scheduled to be held next month.

● The daring young man on the flying machine is Dr Magnus Pyke, Secretary of the British Association for the Advancement of Science, currently to be seen on British television, advertising the appearance of a new partwork about science. Actually his machine amounts to nothing more than a pair of roller skates and a handful of bricks, so nobody seriously expects Pyke to fly. The aim is simply to demonstrate the principle which causes a jet to move—equal and opposite reaction stuff which

comes into play when Pyke throws the bricks in one direction and rolls off in another. The BA Secretary, who always makes entertaining radio or television, says the demo fits neatly his function as an explainer of scientific thought to a mass audience. So it's an important roll.

● The recent death in a London hospital of a patient suspected to be suffering from the virus disease Lassa fever highlights the growing problem of importing exotic and often dangerous diseases into Britain.

Ever since its discovery in Nigeria in 1969, Lassa fever has been viewed with trepidation by health workers. The four epidemics which have occurred in West Africa since 1969 have all been marked by a high death rate among hospitalised cases and by a high risk of infection by close contact, which puts nurses, doctors and relatives caring for the patients at great risk from virus-laden blood, urine and other body secretions.

There have been several cases of Lassa fever imported into Britain, the most recent being earlier this month, when a doctor returning from Kano in Nigeria, died of Lassa fever in the London Hospital for Tropical Diseases two days after his arrival in the UK. As with many tropical diseases, the main problem is a correct diagnosis, especially in Britain. Lassa fever in particular starts with a slowly mounting fever followed by headache, backache and nausea, and unless the doctor is aware that the patient has recently arrived from West Africa, where the disease is endemic, it is almost impossible to diagnose.

● GALLOPING inflation coupled with the slump in capital values of investments has finally forced the closure of one of the oldest established biomedical laboratories in Britain, the laboratories of the Lister Institute of Preventive Medicine in Chelsea Bridge Road, London.

An extra £300,000 a year at current prices would be needed to keep the laboratories in business. But at the end of last year, it was announced that it had been impossible to secure sufficient new funds to prevent the laboratories from closing in 1975.

Warning notes have been sounded over the past few years and the research effort had already been reduced to a level which the Governing Body regarded as tolerable only in the very short term. The laboratories in Chelsea Bridge Road were opened in 1898 and have been in continuous use for the 77 years of their existence. Over the years the institute has made important contributions to the understanding of infectious diseases and parasitology, as well as of basic chemistry and biochemistry.

More than just preparing

from Vera Rich, London

THE launching of the two-man spacecraft, Soyuz 17, and its link-up with the orbiting space station, Salyut 4, should not be considered simply as a preparation for the forthcoming Soyuz-Apollo project. So said Major-General Georgii Beregovoi, Head of the Cosmonaut Training Programme, at a press conference given after the successful link-up. On the contrary, he says, the joint Soviet-USA missions will merely be a part of the Soyuz programme, "a chance to broaden the range of tasks which can be solved using the Soyuz craft".

Although this comment may be interpreted as an echo of the confidence which the successful link-up has evoked in the Soviet space planners after a year notable for its setbacks, there is more to it than simply post-launch euphoria. The primary aim of the current mission, said Beregovoi, is to test out a new control and life-support system. According to the TASS data, this involves approximately normal atmospheric pressure and temperature in the cabin, representing a return to standard Soviet procedure after Soyuz

16, which with its reduced cabin pressure and increased oxygen content was a compromise with US practice.

The control system referred to is presumably the new autonomic navigation system, based on the use of ionic sensors and permitting the station to be orientated with respect to the Sun, Moon and planets in various regimes of orbital flight, and which also includes a device for orientating the spacecraft with respect to the earth in conditions of minimum illumination above the night side of the planet.

Nevertheless, even if Soyuz 17 is not a direct preparation for the Soviet-US mission, its success or otherwise must surely affect the confidence of the space planners of both nations in the outcome of the joint project. □

The Shtern case

The case of Dr Mikhail Shtern, formerly Director and Senior Consultant in the polyclinic of the Vinnitsa Provincial Endocrinological Health Centre has, during the course of pre-trial investigations and the trial itself, caused considerable reaction in the West, which has not passed unnoticed by the Soviet authorities. In the pre-trial investigations, the charges against Dr Shtern ranged from dissi-

dence to the murder of child patients. Apparently as a result of the protests, and also because it proved impossible to find witnesses to substantiate the murder charge, when the case finally came to trial the only charges Dr Shtern faced and those for which he was duly sentenced were those of "swindling" (charging for medical treatment) and "the taking of bribes".

Although these are clearly less serious charges than murder (or, in a Soviet context, dissidence), they seem intended to take Dr Shtern's case outside the competence of such organisations as Amnesty International which undertake to deal with cases of "prisoners of conscience". In the past 18 months, how every, there have been several cases of would-be emigrants to Israel facing criminal charges and, in Dr Shtern's case it was admitted on May 29, 1974 by the Procurator of the Investigations Department, V. Kravchenko that the preparation of an accusation against Shtern was connected with the desire of his family to emigrate to Israel. Accordingly, those who have been campaigning for Dr Shtern have shown no signs of being put off by the circumstances of his conviction. New letters of protest are being prepared, seeking a reversal of the verdict and sentence. □

THE Israeli Government has set up a Ministerial Committee for Science and Technology to tackle problems of research and development, and to determine whether activities in these fields are in line with the general policy of the government.

The scientific community in Israel feels that the establishment of the new committee is long overdue. Scientific and technological activities in the country have expanded extensively during the past five years; mainly because of governmental support its share in total civilian research and development and education investments was 69% in the fiscal year of 1973-74. This share increases when investments in defence research and development are included, since these are totally covered by the government.

The overall investment in research and development and higher education—both civilian and defence—reached a total sum of IL 1,500 million. There is, however, growing public criticism because of what is felt to be a lack of central policy in allocating government and public funds to achieve more direct and positive results. For instance, it is felt here that universities—which are private institutions—are supported too heavily by the state, whereas elementary and secondary schools suffer from lack of funds and teachers. It is also felt that a good deal of the

money spent on civilian research and development yield few benefits to the country in these trying days.

Israel is blessed with a 'brain influx', an inflow of professional manpower. An exceptional phenomenon for a small and developing country, this influx consists of local university graduates, of Israeli researchers who return home after working abroad for several years (mostly in the USA), and of

New Israeli science committee

from Kapai Pines, Jerusalem

scientist-immigrants from North and South America, Europe and lately from the Soviet Union.

Alas, the blessing might become a misfortune if this manpower is not used to best advantage. A central policy is needed and it is hoped that the new Ministerial Committee for Science and Technology will also tackle this problem.

The committee's tasks include: (1) ratification of the general policy of the state for the advancement of scientific research and technological development and the application of

them to meet the economic and social needs of the country; (2) deciding the goals of the state's budget in the fields of research and development, and its priorities; (3) defining inter-ministerial procedures which will assure coordination of all government operations in science and technology; (4) rectifying any changes in the organisational layout of the research carried out in government laboratories; (5) confirming appointments of Chief Scientists in state ministries; (6) confirming any other functions in the fields of science and technology which the committee will think fit.

To stimulate the committee and to give emphasis to inter-ministerial problems, it is composed of ministers whose ministries engage in scientific and technological functions: The Prime Minister (chairman), a minister without portfolio (vice-chairman), and the ministers of finance, defence, health, education and culture, agriculture, commerce and industry, interior, housing and transportation. The coordinator of this committee is the Director-General of the National Council for Research and Development, Dr Eliezer Tal.

● Professor Saadia Amiel, one of Israel's leading atomic scientists, has been appointed to the strategic planning division of the Defence Ministry, according to a report in the *Jerusalem Post*.

North American diary

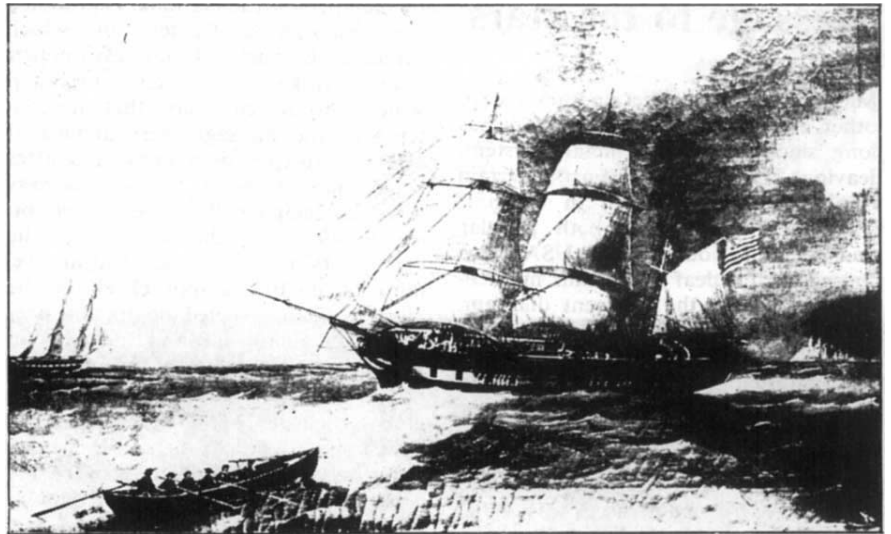
from Angela Croome

THIS month off an island in Hudson's Bay and about 2,000 miles from anywhere else an American adventurer plans to bore a hole through some six feet of sea-ice and with heavy lifting equipment hoik to the surface a New Bedford whaler that sank there 100 years ago. The assumption is that the hull would remain intact during this rough-and-ready recovery but if not, Alexander Byron, the New Bedford restaurateur behind the scheme, is equally confident of cobbling it together on the spot and sailing the ship back to its Massachusetts home port. All this in the polar night with temperatures down to -22°C .

He is offering similar caesarean treatment for the remains of the two Knight expedition ships of 1719, the wrecks of which have also been found on the bottom of the Eastend harbour of Marble Island. These were a British sloop and frigate chartered by the Hudson's Bay Company through the enthusiasm of its lately retired and nonagenarian 'governor' of North American depots, Sir James Knight, who was convinced of a river of gold at the end of the equally mythical North-west passage to the Pacific. The expedition got no further than Hudson's Bay, lost both ships in a violent storm and perished lingeringly after three years cut off from the mainland on Marble Island. The Knight expedition is one of the most pitiful tales in the whole gamut of polar exploration, and there would be unique archaeological and expeditionary interest in the actual remains of this early polar voyage. Most of this evidence is bound to be lost by the recovery methods proposed at present. One can imagine the outcry there would be in Britain if a 250-year-old expedition ship and its contents were to be rudely winched from the sea bed without regard to conservation, recording or any other archaeological techniques.

Not so in Canada—although two distinct licences have to be obtained from government departments before any project may be pursued in the Northern Territories. Admiration of the old North American virtue of "can-do" still, it seems, outweighs the value put on the epic past.

● The campuses are taking the lead in concern for the energy environment in a way that one looks for in vain outside America. While British government offices have only recently introduced a 60°F heat limit, all the professors' houses I visited in the USA and Canada this winter had their temperatures set at something less than



A New Bedford whaler of the type that Alexander Byron may fish up.

60° , and had been so at least since the beginning of last winter.

Walking is becoming quite fashionable at Princeton, Harvard, McMaster, Sarah Lawrence and other suitable centres though a nasty mugging of a sociologist's wife in Cambridge recently has discouraged movement on foot after dark. Bicycles are widely replacing cars on campuses too—sometimes with rather grisly results because American academics are wedded to the idea that cycling is *sportif* and that a bicycle is not 'traffic'. The common regulations of lighting and flow direction are scarcely observed and it is a disconcerting experience indeed when driving at night to meet an oncoming cyclist, head well down, with no lights and on the wrong side of the road.

This approach contrasts strongly with that of the motorist. The reduction in traffic deaths during last winter's fuel crisis was so remarkable that a speed limit of 55 miles an hour has been adopted permanently for all roads in the USA. The amazing thing to a European is that everyone conforms, and in the most orderly and disciplined fashion. The massive overpowered machines, still gobbling gas at a rate of about 15 miles to the gallon, progress on the expressways in a well spaced and stately stream at an average $52\frac{1}{2}$ miles an hour. Driving is emphatically no longer a blood-sport in the United States—as it is (one realises anew) for Europeans, especially the British, and, indeed, for Canadians. There is no nonsense with a national speed limit or courtesy between drivers on the six lanes of the Queen Elizabeth Highway, supposedly the world's most travelled road, which connects Toronto to the industrial heartland of the United States.

● Cholesterol is the other general campus preoccupation. While women

and children tuck in as ever to butter and eggs, the middle-aged husband resolutely sticks to dietary margarine and a one-egg-a-week regime. And, of course, he 'jogs'—typically a mile a day and in appropriate dress. The whole thing is taken so earnestly, with or without medical evidence of enhanced cholesterol levels, that one wonders whether the cholesterol scare is a subtle conspiracy by Women's Lib to chastise the profligate male.

● Vasectomy—an operation only really popular in England among policemen apparently—is both widely performed and discussed. "Did I know many men who had had the operation?" Well, I do now. Perhaps this is another reflection of the leadership of the campuses in environmental concern.

● But there is a freemasonry among early morning exercisers that has much to recommend it. A Washington journalist who shares a taste for tennis rather than jogging with one of the country's most dedicated astrophysical experimenters, found himself the repository of speculation about why the scientific press neglected the amazing results emerging from the new generation of super-large radio interferometers. The 90-mile baseline proposed for this type of work when—and now if—Jodrell Bank's out-station at Meifod, Wales, were built is piffling in comparison with the power of interferometer systems using baselines which rely on telescopes continents apart. Such an arrangement is already in use synchronising telescope observations in the USA and the Soviet Union. The next stage, a Moon–Earth link-up, would give a baseline 250,000 miles long and an analytical power in proportion. Already the infrastructure of cosmic processes in undreamt-of detail is rolling off the computers on which this instrumental approach depends. □

Message to the stars

from Ian Ridpath

MANKIND'S first deliberate message to other civilisations among the stars has long since left our planetary system, leaving behind it on Earth certain rumblings about the way in which it was done. The media, both popular and scientific, outside the USA have turned such a deaf ear to this momentous event that the adjacent diagram, transmitted by the slower methods that remain conventional on Earth, may be the first detailed description of it to reach many parts of the community.

The 1,679 part message was transmitted in 169 seconds from the 1,000-foot Arecibo radio telescope in Puerto Rico. The frequency used was 2,380 MHz (wavelength 12.6 cm)—not one of the standard lines suggested for interstellar communication, but instead the top end of Arecibo's radar astronomy facility that is being used for projects such as the detailed mapping of Venus.

Writing in *Nature* for October 5, 1973, Frank Drake and Carl Sagan of Cornell University noted that the telescope's new capability would allow it to communicate with an identical instrument anywhere in the Galaxy. The Arecibo message, transmitted after a re-dedication ceremony following the resurfacing of the Arecibo arch, can only be seen as an attempt to demonstrate the truth of their proposition.

The key to the message is that it breaks down into a grid, 23 characters by 73. The diagram shows the picture that can be built up from it. Reading from top right (a tricky start) the pictogram describes in binary form the numbers one to ten as a kind of lesson to establish the language of the following message. Below this top row of numbers is a group displaying the atomic numbers of hydrogen, carbon, nitrogen, oxygen and phosphorous.

Next, the message uses this information to describe the molecular components of DNA and on lines 32 to 46 actually depicts DNA's double-helix structure. The central core is the number 4,000 million—roughly the number of characters in the genetic code.

All this adds up to a description of the chemical basis of terrestrial life: next comes a cryptic human (Ned Kelly in gumboots?) with an indication of his height to the right (14 wavelengths of the transmission) and on the left the approximate number of the human population (4,000 million again).

On the next line is a sketch of the solar system, with below it a representation of the Arecibo telescope itself, pointing downwards to a number that roughly describes its diameter.

This interstellar IQ test was devised

by members of the National Astronomy and Ionosphere Center, of which Arecibo is part. Radio astronomer Frank Drake of Cornell University, which runs Arecibo, says that the contents of the message were arrived at after exhaustive discussions, and after being 'market tested' to see how easy it was to decipher. Some people, doubtless familiar with the workings of the originators' minds, managed to unravel most of it. But a spot check in the *Nature* office revealed no intelligences (native or alien) that could decipher it. Are interstellar IQ tests culture fair?

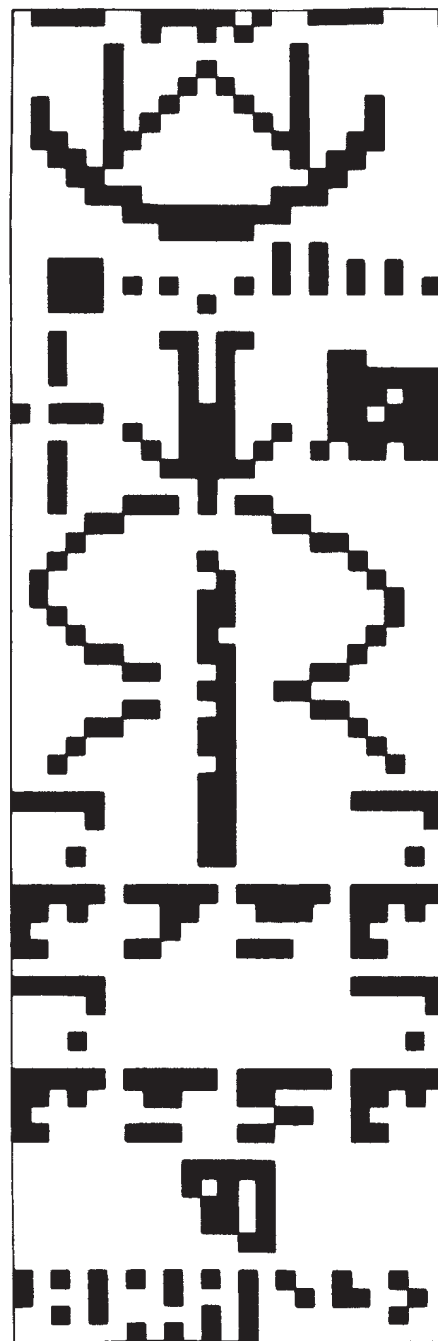
The message was beamed at the globular cluster M13, which is 24,000 light years away. At that distance the radio telescope beam just covers the cluster's 300,000 stars. Astronomer Carl Sagan estimates that there is a one in two chance that there will be a civilisation there to receive it. The signal frequency was modulated to correct for the motion of the Earth in space, although other radio astronomers have pointed out that just such a Doppler shift on an interstellar signal would give valuable information about the orbit of the sending planet and its axial spin, as well as its size.

The single transmission of such a message cannot be regarded as a very serious attempt at interstellar communication; more likely, it will serve as an example to boost the funding chances of those who wish to listen for similar messages coming to us from other civilisations. No prior news of the Arecibo message was given, even to delegates who discussed interstellar communications at the previous month's International Astronautical Federation meeting in Amsterdam.

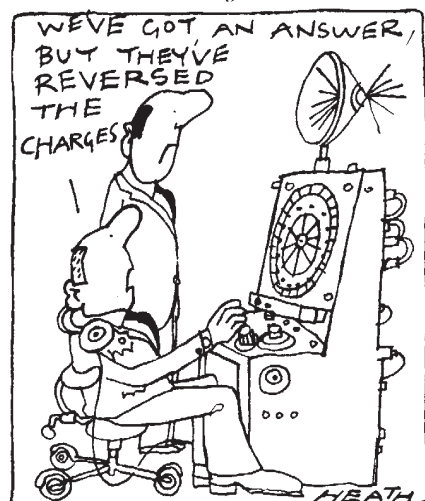
Yet at the 1971 International Meeting on Communication with Extraterrestrial Intelligence (CETI) the delegates concluded that such undertakings were best done "by representatives of the whole of mankind".

Drake now says that he did not consider the Arecibo message a major enough event to require international cooperation. But Tony Lawton, a member of the CETI standing committee of the International Academy of Astronautics, thinks that it has established "a very nasty precedent" and is frankly surprised that it has been done at all without prior international discussion and approval. He says that "now there is nothing to stop any nation from transmitting any signal, anywhere, to any spot it chooses. A little warning, a little consultation, would have been better."

The critics still have time to influence matters for the future. The Arecibo message is planned to be repeated when telescope time permits, although no dates have yet been chosen.



Above: the message. Below: answer?



Courtesy The Sunday Times

news and views

ASTROPHYSICISTS experience biennial urges to congregate in the Texas Symposia on Relativistic Astrophysics (irreverently, Gee-whizz meetings). Recently, five hundred participants assembled in Dallas where from 16–20 December almost fifty scheduled speakers and many more in so-called workshops, summarised progress since the last meeting (*Nature*, **241**, 169; 1973). Necessarily incomplete impressions follow.

Cosmology opened the proceedings. Reeves (SEP-CEN, Saclay), noting the embarrassingly large interstellar deuterium abundance, remarked "Anybody who wants to believe in the big bang producing a closed universe had better invent a way of making deuterium; and that's not easy, I can tell you". Colgate (University of New Mexico) suggested supernova shock waves as a site, but the general impression of a severe restraint remained.

Gunn echoed this theme in his State of the Universe message (traditionally reserved for Caltech and its satellites). Gunn and colleagues have assembled many arguments (stellar, nuclear, inferred densities, and so on) pointing to an open Universe (see *Astrophys. J.*, **194**, 543; 1974). He and Oke, with seventeen new redshift-magnitude points for $z > 0.25$, produced a "classical test" which disconcerted many. Tinsley (University of Texas) later presented spectroscopic evidence for giant-star dominated ellipticals. The implied evolutionary dimming necessitates reductions in observed values of the Sandage variable q_0 by 1 to 1.5 or more. Thus emerged a large, negative value for the true q_0 ; alternatively, a large positive value for Einstein's disinherited Λ , the cosmological constant. When Fowler (Caltech) arose to demand if this were so, Gunn retreated to the sanctuary of probable errors. Perhaps cosmologists should always state "These are the opinions upon which I shall base my facts".

Ostriker (Princeton University) supported the density estimates when discussing halos of galaxies. Galactic disk stability requires a massive halo. Elsewhere, masses from mass-luminosity ratios increase with scale up to the 'virial masses' of clusters. New data obtained with Spinrad reveal extended red halos around edge-on spiral galaxies; the now respectable virial masses still fail to close the universe.

G-wizardry at Dallas

from John Faulkner

The observations of neutral currents in weak interactions have had dramatic effects on supernova theory. Schramm (University of Chicago) summarised implications for collapsing iron-nickel core models (descending from the work of Fowler and Hoyle). Freedman's suggestion that neutrino scattering cross-sections will scale as the square of atomic number may be the key permitting central collapse to neutron stars of black holes, while producing mantle ejection. Arnett (University of Illinois) has followed evolution from well understood helium burning to the hydrodynamic phase of neutron-star formation. Wilson (Lawrence Livermore Laboratory) and Bruenn (University of Florida) found shock waves in collapse calculations. Whether black hole formation or nucleosynthetic eruption would ensue was unresolved. Later Weinberg (Harvard University) presented a virtuoso account of the furore in physics and astrophysical consequences. Neutral currents, charmonium and a possible unification of strong, weak and gravitational forces at large energies were tantalisingly dangled before a mesmerised audience.

The mysterious gamma-ray bursts were discussed by Strong (Los Alamos) and Cline (Goddard). In one doubly observed event Vela detectors caught only the tail-end; earlier onset data should thus be regarded with caution. Ruderman (Columbia University) regaled listeners with a scintillating review of the flood of unconstrained theories, more numerous than the events themselves, involving black holes, neutron stars, antimatter, white holes, novae, ablating relativistic dust grains. "Nothing is too wonderful to be true, but there is not yet much else to support such a host of clever inventions."

Developments in relativity theory were reviewed by Ellis (University of Cape Town) and Trautman (Warsaw). Ellis emphasised that Bianchi (spatially homogeneous but anisotropic) cosmologies can have very different singularity

properties from those usually considered. Matter can originate in spatially inhomogeneous regions, cross prediction horizons, and end up in spatially homogeneous regions. Some charmingly named universes (for example, "Whimper-bang-whimper") await discovery; it was noted with evident relish that the steady state begins not with a bang but a whimper. Teukolsky (Cornell University) reviewed black hole perturbations, where Chandrasekhar has developed some unifying formalism. Practical gravitational radiation was almost conspicuous by its absence (a noted observational characteristic?). The anti-Weber climate (*Nature*, **250**, 287; 1973) has had curious backlash effects on other work involving gravitational radiation. Spectroscopy of HZ29 showing evidence for the predicted mass transfer was first dismissed by an organiser as the work of self-evident cranks. Hawking (University of Cambridge) penitently confessed that black holes were white hot, emitting through quantum effects, radiation which makes small holes ($\lesssim 4 \times 10^{14}$ g) evaporate in less than the age of the Universe.

X-ray day dawned. A promise by Giacconi (Harvard University) that the meeting would now become interesting, was amply fulfilled after his talk when Pounds (University of Leicester) showed Cen X-3 data from the UK-5 satellite with a strong dip at phase 0.62 in each binary period. This was regarded as excellent support for the extended Pringle wake model which Giacconi had presented. The day ended with mad scrambles between two simultaneous X-ray workshops involving no fewer than 38 speakers.

Strittmatter (University of Arizona) was permitted 25 minutes to cover QSOs (how are the mighty red shifted!). After suggesting that much of the effort on BL Lac had been misdirected, he discussed the increasing evidence for multiple absorption redshifts and line-locking (for example CIV in 3C191) agreeing with Scargle's predictions. A new $z=3.2$ QSO was announced which, like OH471, appears neutral or reddish. It reopens the question of selection effects once more and emphasises the importance of working systematically through Hazard's suitcase-full of unbiased accurate positions. Small scale radio structure was described by Cohen (Caltech). Apparent linear 'expansion'

velocities up to $\sim 10c$ are seen, and in 3C273 three independently varying components simulating motion can be ruled out. Four or more components are required for the 'theatre marquee' effect to work, but the existence of 'expansions' only is a puzzle.

The organisers, perhaps with the misguided intent of reserving the finest vintages till last, arranged three of the most important papers for the final chaotic afternoon. The Brans-Dicke theory received two almost mortal blows. Hill (University of Arizona) finds the solar oblateness at a time when confusing excess brightness is absent to be lower than Dicke's value by more than an order of magnitude. It agrees with expectations for a uniformly rotating Sun to within 25%. Consequently, Mercury's perihelion shift has a negligible contribution from a quadrupole gravitational field and supports Einstein over Brans-Dicke.

One fascinating by-product emerged: old Sol is breathing (pulsating seems too strong a term) in a number of modes including the gravest, ~ 54 min (geophysicists note!). Fomalont (NRAO, Green Bank) reported results of the microwave radiation bending from three QSOs with angular positions close to the Sun's path in April. The result, 1.015 ± 0.011 times the Einstein prediction, is not consistent with a scalar coupling constant $\lesssim 23$. Finally, Taylor (MIT) announced that the binary pulsar 1913+16 is indeed showing apsidal motion of 4.0 ± 1.5 degree yr^{-1} , consistent with general relativity and suggesting two compact components of ~ 1.3 solar masses each.

The meeting was a great success, if at times in spite of the arrangements by the 19-strong organising committee. I thank those many speakers who, guided no doubt by self-preservation, provided summaries of their talks.

Ethiopian fossil hominids

from a Correspondent

WITHIN the last decade and a half field work in east Africa has had an extremely important impact on all aspects of early man studies. The initial impetus for east African field studies came of course from Olduvai Gorge but subsequently successful field programmes at East Rudolf in northern Kenya and in the Omo River area of southern Ethiopia have been no less important and productive. In late 1973 a new area was added to the east African inventory of fossil man sites. This new area on the Hadar River, a tributary of the Awash River, in eastern Ethiopia has produced, to date, a total of nine fossil hominid specimens.

Geological and palaeontological studies in Plio-Pleistocene deposits in this area have been in progress since 1967. These studies have resulted in a brief report (Taieb, Coppens, Johanson, and Kalb, *C.r. hebdomadaire Séances Acad. Sci. Paris*, 275, August 16, 1972) of geological sequences and palaeontological remains from several sites in the Awash area. Formations at Haoua-Lédi and at Leadu have been closely correlated, on the basis of faunal remains, with deposits in the Omo River valley dated to between 2-3 Myr. Deposits at Hadar may be a little older and have been faunally correlated with the lower levels at Omo, perhaps, in absolute terms, at about 4 Myr. This latter region yielded, in late 1973, five hominid fragments: an associated right distal femur and proximal tibia, proximal left and right femora and a left

temporal bone.

A full description of this material has not yet been published but a brief report (Taieb, Johanson, Coppens, Bonafille and Kalb, *C.r. hebdomadaire Séances Acad. Sci. Paris*, 279; August 26, 1974) mentions that the femoral neck is flattened anteroposteriorly and is oval in section; the distal femur, said to be "très humaine", has an elongated and flattened external condyle and a deep intertrochanteric fossa. The authors, in stressing the very small size of this post-cranial material, conclude that the height of this individual from Hadar was less than that postulated for one of the individuals from Sterkfontein. Most estimates of the height of STS 14, for example, based on reconstructed femoral length, do not exceed $4\frac{1}{2}$ foot. Perhaps the most interesting and significant feature of the distal femoral fragment is not mentioned in the text but is apparent from the accompanying photograph. It seems clear that the natural anatomical orientation of this femur would include a relatively high degree of shaft obliquity. This angulation, which would permit the knees to approximate the mid-line during upright stance, is an important hominid characteristic and distinguishes the femora of this group from those of other primates. Although this material was not formally attributed to any taxa the morphology of the temporal bone, which is highly pneumatized, was said to resemble that of the robust australopithecines.

Although the hominid nature of at

least some of this material would seem confirmed the dating is not quite so clear. The geological sequence in the Hadar area seems well worked out but the fact that this fossil material was not found *in situ* but was scattered over a considerable area of the surface has led the researchers to express some doubt over the exact position of its original location within the sequence. Thus the "niveau à hominidés" designation in the geological column is followed by a query (?).

In October, 1974 further hominid remains were reported from the Hadar area. These remains, consisting of four jaw fragments, include a complete maxillary arcade with only the incisors and one canine missing. The first report of this material, at a press conference in Addis Ababa on October 25 (see *The Times*, page 6; October 26, 1974), included the following statement:

"These specimens clearly exhibit traits which must be considered as indicative of the genus *Homo*. Taken together, they represent the most complete remains of this genus from anywhere in the world at a very ancient time.

"All previous theories of the origin of the lineage which leads to modern man must now be totally revised. We must throw out many theories and consider the possibility that man's origins go back to well over four million years."

These are rather strong statements and may be examined on several levels.

First, although the reported potassium/argon date of 3.01 ± 0.25 Myr for a basalt in the Hadar area may well be valid this fossil material, as with the 1973 specimens, was not recovered *in situ* but was collected on the surface. Thus, the specimens' relationship to the dated level cannot be known with complete certainty. In an area such as the Awash ephemeral stream activity can move material over considerable distances and the absolute dating of surface finds must be viewed with some scepticism. Second, no morphological or metrical data have been presented so that the attribution of this material to the genus *Homo* cannot be fully evaluated. It should be pointed out, however, that the available photographs show certain features of the canines, premolars and molars which may not be entirely consistent with current definitions of that genus. Third, the statement that "all previous theories" of hominine origins must now be revised in the light of this material is simply untrue. The results of earlier field work at East Rudolf, based on material found *in situ* and in a well-dated context, have already supported an early origin of the genus *Homo*.

The final point is not, however, the

ultimate or intrinsic scientific value of these fossils; the data are at present inadequate to make such an evaluation. The point is that questions of taxonomic attribution, phylogenetic position and, ultimately, scientific importance must follow upon careful comparative studies and complete anatomical and metrical evaluation. Such a reversal of priorities, with its consequent and inevitable de-emphasis on necessary laboratory procedures is completely inconsistent with current scientific method and theory.

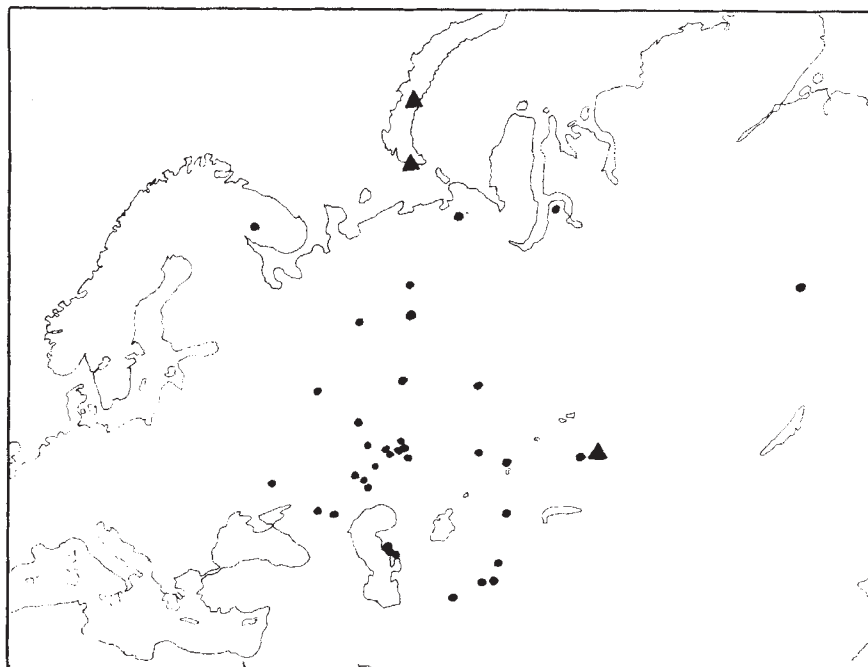
Stagger to deceive

by David Davies

THE problem of detecting and identifying underground nuclear weapons tests has been around for nearly twenty years. In 1958 there were high hopes that seismic techniques would prove adequate to monitor tests down to a few kilotons without any territorial intrusion. A year later these hopes were dashed when the big-hole theory was wheeled out by the American weapons laboratories—fire an explosion in large enough a cavity and its seismic signal is almost negligible. In the early sixties hopes rose and fell as the Americans, British and Russians played, inconclusively, with numbers of on-site inspections and black boxes. In the late sixties hopes began to rise as seismic methods improved and a new technique was developed based on the relative excitation of surface and body waves. In the early seventies hopes rose to a peak when there was talk on an underground test ban but there was widespread disillusion when the nature of the ban (on explosions above 150 kilotons from a date in 1976) became clear.

Now a new problem emerges. In this week's *Nature* (page 242) Kolar and Pruvost report for the first time in the open literature on techniques that could be adapted by a country wishing to evade a strict ban.

It has been widely accepted for many years that a test fired while the Earth was ringing from a really major earthquake (of the sort that occurs only once or twice a year) would be impossible to detect. This presents a rather risky prospect to the determined evader: a more measured scenario in which the evader fired at a time of his own choosing would obviously be more desirable. Yet to do this some way had to be found to increase the excitation of surface waves relative to body waves. This could be done, argue Kolar and Pruvost, by firing a multiplicity of shots, one of which would be the device under test.



The dots are the locations of presumed 'Plowshare' explosions. The triangles are Soviet test sites.

The relative excitation of surface and body waves is not actually changed by this technique, but since the techniques for measuring the excitations are relatively crude (necessarily; signal-to-noise ratio is frequently no greater than unity) it is possible to deceive the measuring process. The dominant period of a body wave is 1 second and the rules for determining excitation specify a measuring interval of three or four seconds so the apparent magnitude from a string of explosions over many seconds is of necessity low. On the other hand surface waves have a dominant period of 20 seconds, so provided the time delay from first to last explosion is kept smaller than about 10 seconds, all surface waves add in phase. Thus the apparent enhancement.

Many other precautions will have to be taken to avoid satellite and ground monitoring, but Kolar and Pruvost believe that these can be satisfied and thus that there will be serious doubt if not complete misreading in the monitoring country.

Should such ideas for deception be surfaced, revealing a mistrust of Soviet intentions? Most certainly; they have been around in the grey area between secrecy and openness for several years, and it is now necessary to take them seriously so that in any future debate on test-ban technology there will be no surprises. It will not escape the reader that the authors work for a laboratory charged with research into nuclear weapons and therefore presumably with a certain interest in forestalling any total ban on weapons testing. But no

doubt many seismologists elsewhere with a different point of view will now try to crack this new problem.

● In a related field, the figure shows the enormous extent of Soviet 'Plowshare' nuclear explosive activity. Each point represents an event reported in the open seismic listings which can reasonably be taken to be an explosion for some peaceful purpose. Yields vary from a kiloton or less to more than 100 kilotons, and the sparse literature from the Soviet Union suggests uses ranging from canal-building to oil field engineering.

Sizing the RNA tumour virus genome

from Benjamin Lewin

WHEN RNA is extracted from the C-type RNA tumour viruses, the major components sediment at 60-70S and 4-5S. After denaturation by heating or by treatment with DMSO, 60-70S RNA sediments in neutral gradients at 30-40S. The apparent $10-12 \times 10^6$ daltons of the 60-70S RNA thus seem to comprise subunits of about 3×10^5 daltons; given the uncertainty inherent in this determination of molecular weights, there might be two, three, or four subunits in the 60-70S complex. A question that has remained unaltered for some time is whether the subunits in a virion all are identical (so the genome would be polyploid) or whether they carry different sequences (giving a haploid genome). The genetic

information contained in a single subunit would be sufficient to code only for about 300,000 daltons of protein, which compares with the total weight of known viral proteins of about 3,650,000 daltons.

The presence of two types of subunit in 30–40S preparations of Rous sarcoma virus (RSV) RNA was first reported by Duesberg and Vogt (*Proc. natn. Acad. Sci. U.S.A.*, **67**, 1673–1680; 1970) when they electrophoresed the RNA on gels; these are the more slowly migrating (larger) *a* subunit and the smaller *b* subunit. The size of the class *a* subunits is in the range $2.4\text{--}3.4 \times 10^6$ daltons and that of the *b* subunits is about $2.2\text{--}2.9 \times 10^6$ daltons (Duesberg and Vogt; *J. Virol.*, **12**, 595–599; 1973). But Martin and Duesberg (*Virology*, **47**, 494–497; 1972) then showed that the 60–70S RNA extracted from cloned RSV particles derived from clonal populations of infected cells carry only the *a* subunit. In a further report, Duesberg and Vogt (*Virology*, **54**, 207–219; 1973) showed that the *a* subunit of cloned Schmidt–Ruppin strain of RSV is slightly larger than the *a* subunit of the cloned Prague RSV strain.

Different (noncloned) strains of avian sarcoma virus have different ratios of *a/b* subunits. Avian leukaemia virus particles, however, contain only *b* subunits. Since the sarcoma virus can transform chick fibroblasts whereas the leukaemia virus is unable to do so, this observation led to the concept that the *a* subunit may carry information necessary to accomplish transformation and that this sequence is missing from the leukaemia virus genome. Consistent with this idea was the observation of Duesberg and Vogt that transformation-defective variants of avian sarcoma virus all possess only the class *b* size of subunit. The derivation of *b* subunits from *a* subunits might therefore be explained by the occurrence of a deletion in the *a* subunit which removes information essential for transformation. If all the subunits in either size class are identical, then a single event would be sufficient to generate the defective viruses; but if the subunits represent different sequences, presumably a deletion would have to take place in each type of molecule in order to generate the size change between sarcoma and leukaemia virus.

Two approaches have been taken to determine the complexity of the RNA genomes and these have yielded contradictory results. Chemical analysis suggests a polyploid structure for the avian RNA virus genome. After digesting the RNA of the Schmidt–Ruppin strain of Rous sarcoma virus with T1 ribonuclease and separating the oligonucleotides on two-dimensional gels, Billeter, Parsons and Coffin (*Proc.*



A hundred years ago

THE ZODIACAL LIGHT.—On the evening of Sunday last, the 24th inst., a surprisingly bright display of this as yet problematical phenomenon was exhibited. There was a repetition on the following evening, but in a less favourable sky. The light had the usual yellowish or pale lemon tinge of the more notable exhibitions in these latitudes. The axis of the light appeared to pass λ Piscium, and the vaguely-defined apex was situate somewhere about 19 Arietis, but it was not possible to locate it with anything like precision. The light was broad and of a deeper, perhaps, ruddy tint near the horizon. The display to which we have adverted, excelled in brightness any that has been witnessed in the neighbourhood of London for many years. It appears very probable that opportunities for favourable application of the spectroscope may be afforded in the dark evenings of the present and following months.

From *Nature*, **11**, 249, January 28, 1874

natn. Acad. Sci. U.S.A., **71**, 3560–3564; 1974) obtained roughly twice the number of spots generated by 28S rRNA. By determining the molar yield of 11 of these spots, they were able to suggest a complexity for the viral genome of about $3.4 \pm 0.9 \times 10^6$ daltons. A similar estimate was made by Quade, Smith and Nichols (*Virology*, **61**, 287–291; 1974) for the RNA of the Prague strain of RSV (3.3×10^6 daltons). These estimates would suggest that the genome is polyploid, since the sequence complexity of the viral RNA is about the length of a single 30–40S subunit molecule.

That *b* subunits are derived by a deletion of *a* subunits is suggested by the chemical and hybridisation analyses reported by Lai, Duesberg, Horst and Vogt (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 2266–2270; 1973). Comparing the T1 ribonuclease digests of the RNAs of avian sarcoma viruses and transformation-defective derivatives showed that all the large spots of the *b* subunit are found in the digest of the *a* subunit; but the *a* molecules contain one or two additional spots not found in the *b* digest. The labelled cDNA prepared from the *a* subunit hybridises with about 70% of the *a* RNA when saturation is reached; it hybridises with 60–65% of the *b* RNA, a result which would be consistent with the omission from the *b* RNA of a sequence present in the *a* RNA. Neiman *et al.* (*J. Virol.*, **13**, 837–846; 1974) have compared the

RNA genome of the Prague strain of RSV with a spontaneous transformation-defective mutant derived from it by hybridising these viral RNAs with excess chicken DNA of normal embryos and sarcomas. The transformation-defective strain seems to lack a sequence present in the parent sarcoma virus.

Hybridisation experiments have been used also to measure the complexity of the 60–70S RNA of RSV. When labelled cDNA prepared from the viral genome is annealed with an excess of RNA, the reaction rate depends upon the complexity of the RNA. Taylor *et al.* (*J. molec. Biol.*, **84**, 217–221; 1974) reported a sequence complexity of 9.3×10^6 daltons, which corresponds to about three different subunits if each subunit is about 3×10^6 daltons. This clearly suggests a haploid genome. No reconciliation between the chemical and hybridisation analyses is at present apparent.

What is responsible for maintaining the association of subunits in the 60–70S complex? The denaturation maps recently constructed by Mangel, Delius and Duesberg (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 4541–4545; 1974) suggest that hydrogen bonding at several locations may be implicated. The T4 gene 32-protein binds cooperatively to single stranded nucleic acid and (mildly) denatures both 60–70S and 30–40S preparations. The molecules treated with 32-protein can then be visualised by electron microscopy. In the presence of 32-protein, 30–40S RNA is linear, appears fully coated with protein (thus losing the extensive secondary structure seen in the absence of 32-protein), and has a maximum length (some molecules are shorter, presumably due to degradation) of 2.75×10^6 daltons. The complex of 32-protein with 60–70S RNA consists of several linear molecules held together at different sites, with four to eight free ends per complex. Most of the RNA is coated with protein. The average length is 6×10^6 daltons, which would suggest that the complex includes only two subunits.

Manganese can grow rapidly

from Peter J. Smith

FERROMANGANESE nodules and crusts typically grow on the ocean floor at rates of 0.1–1.0 cm per million years and have Fe/Mn ratios of 0.5–2.0. But Scott *et al.* (*Geophys. Res. Lett.*, **1**, 355; 1974) now report that they have found a manganese oxide (MnO_2) crust with a much higher growth rate, an abnormally low iron concentration and unusual trace element characteristics. The newly discovered crust is thus

atypical but apart from demonstrating just how fast manganese can accumulate under the right circumstances, it also appears to be indicative of hydrothermal processes along active ridge crests as recently proposed in more general terms by Lister (*Eos*, **55**, 740; 1974) and others.

The crust sample in question, which is a 4.2 cm thick deposit of birnessite with a trace of todorokite, was dredged from the median valley of the mid-Atlantic ridge about 5 km from the ridge axis at 26° N. The first hint of its anomalous nature came from a radiochemical analysis of uranium series isotopes. Deep sea manganese deposits usually contain quantities of ^{230}Th and ^{231}Pa far in excess of those required for equilibrium with the parent uranium isotopes, indicating that thorium and protactinium have been taken up from the sea water during the process of manganese accumulation. The decay of these excess isotopes may then be used to determine the rate at which the manganese was produced. But the sample analysed by Scott and her colleagues turned out to be depleted in ^{230}Th and ^{231}Pa ; the manganese accumulation rate had therefore to be measured from the growth of the isotopes towards equilibrium with their uranium parents.

The rates thus determined were, respectively, 13.0 and 25.0 cm per million years, which are held to be in "reasonably good agreement" but which compare with accumulation rates of only 0.1–0.5 cm per million years for nearby samples of 'normal' manganese. This disparity explains in part the unusual trace element composition of the Scott sample, for the concentrations of trace metals removed from sea water are generally the lower the more rapidly the manganese grows. Once the accumulation rate had been determined, therefore, the low observed concentrations of Cu, Ni and Co were hardly unexpected. However, as Ku and Glasby (*Geochim. Cosmochim. Acta*, **36**, 699; 1972) noted, this phenomenon does not apply to uranium, presumably because uranium is incorporated into the manganese in a different way.

Low trace metal concentrations, rapid growth rates and high degrees of fractionation of Mn from Fe are characteristic of both hydrothermal deposits and deposits remobilised from reduced sediments. But what makes a hydrothermal origin the more likely for the Scott sample is the fact that the Mu/Fe ratios of 360–3,600 (the Fe content is as low as 0.01% in places) are much higher than those found in diagenetic continental margin nodules. Other evidence supports the same view, but is more circumstantial. For example, bottom photographs show that the site from which the Scott sample

was dredged is essentially sediment-free; thermal measurements indicate a sharp 0.14° C increase in bottom water temperature over the site; and there is a local magnetic low which could reflect hydrothermal alteration of magnetic minerals in the pillow lavas. Taking all this evidence together, Scott *et al.* conclude that the MnO_2 crust was deposited by a hydrothermal spring in the mid-Atlantic ridge's median valley.

Non-coding regions of messenger RNA

from Pamela Hamlyn

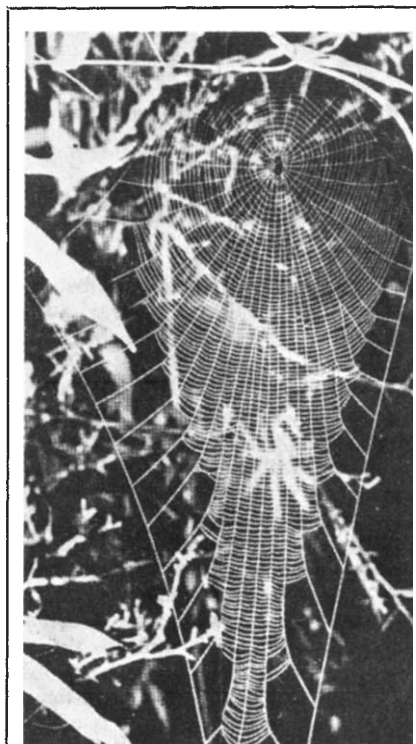
ALL purified messenger RNAs isolated so far have been shown to contain considerably more nucleotides than are required to code for their particular protein. Interest in these untranslated sequences has been centred round the possibility that they may be the site of interactions related to translational control of protein synthesis—a mechanism for which there is much circumstantial but little direct evidence. Part of this non-coding region is accounted for by the poly(A) sequence found in most eukaryotic mRNAs. Despite intensive research, the results of which have often been summarised in these pages, the function of this sequence remains obscure. Current research has shown that the poly(A) region becomes shorter as the messenger 'ages', suggesting that the length of the poly(A) region provides information about the number of rounds of translation possible before degradation of the messenger.

With these ideas in mind, Jeffrey and Brawerman (*Biochemistry*, **13**, 4633; 1974) compared the length of the poly(A) region in messengers from rapidly dividing undifferentiated cells (mouse sarcoma) with globin mRNA from rabbit reticulocytes—highly differentiated cells which do not divide. They confirmed that the poly(A) region becomes shorter with age but suggest that a terminal size is reached. Digestion of polysomes with ribonuclease yields poly(A)–protein complexes, and the authors were able to show that in mouse sarcoma these complexes become smaller and more heterogeneous with time, whereas poly(A)–protein complexes from polysomes synthesising rabbit globin are much more homogeneous. Unfortunately, these studies provide little new information, and consequently the authors have not been able to further elucidate the role of poly(A).

Results of research into the non-poly(A), non-coding regions of messenger RNAs are limited and seemingly contradictory. Nichols and Eiden (*Biochemistry*, **13**, 4629; 1974) have

reasoned that sequences next to the poly(A) region of mRNA might be recognition sites for poly(A) synthetases or else represent termination signals for transcription by RNA polymerase (poly(A) is added post-transcriptionally). They speculate that this would result in a region of homology in the sequences next to poly(A) throughout a population of messenger RNAs.

To investigate this, Nichols and Eiden have prepared ^{32}P -labelled polyosomal RNA from L cells, then digested it with RNase T_1 (an enzyme specific for guanylate residues) and, using poly(U) affinity chromatography, have isolated fragments which contain poly(A) and a few bases at their 5' ends. Sequencing of these bases showed that there was no particular sequence common to all (or even a large proportion) of the messenger RNAs, although more than 50% of the total mRNA molecules contained a single pyrimidine residue next to the poly(A). The authors conclude that there is no recognition site common to all mRNAs in terms of nucleotide sequence.



Web of immature *Eustala* (?) sp. from a forest on the Pacific coast of Columbia. W. G. Eberhard (*J. nat. Hist.*, **9**, 93–106; 1975) describes the construction of this web and an even more elongated one made by *Scoloderus* sp., also in Columbia. Both webs bear a remarkable resemblance to a ladderlike web made by a New Guinean araneid spider and described by Robinson and Robinson (*J. nat. Hist.*, **6**, 687–694; 1972).

Proudfoot and Brownlee (*Nature*, **252**, 361; 1974) have sequenced the 3' end of purified rabbit β -globin mRNA next to the poly(A) region and compared it to the sequence of bases in the same position in mouse immunoglobulin light chain mRNA (Milstein *et al.*, *Nature*, **252**, 354; 1974). They did not sequence the RNA directly but transcribed it into DNA using *E. coli* DNA polymerase I and deoxytriphosphates, one of which was labelled with ^{32}P .

Comparison of the two sequenced regions of each messenger showed that two sequences (7 and 4 bases long) were common to both messengers. As well as this sequence homology Proudfoot and Brownlee suggest that structural homology exists since both the light chain and globin 3' ends can be drawn with loops which are identically positioned and almost the same size in the two mRNAs. Most of the homologous sequence is probably in the unpaired region.

The apparent contradiction between these authors' results and those of Nichols and Eiden will probably disappear as the sequences of the 3' ends of more messengers become available.

Classical relativity in an up-to-date context

from W. H. McCrea

THERE has recently appeared a substantial paper by F. Salzman "Gravitational Field of a Freely Moving Mass" (*Il Nuovo Cimento*, **24B**, 157-188; 1974); the treatment is intended to be that of standard general relativity, however with emphasis on the dynamical rather than the geometrical point of view, and the application is mainly to the case of a Schwarzschild mass. In days when there is so much interest in the gravitational and dynamical properties of black holes, such a study of intimately related problems would be expected to be particularly timely and significant. Indeed, the surprising thing is that the problems have not long ago been fully investigated. Nevertheless, while the paper is of much interest in an exploratory way, it seems that definitive results are scarcely yet achieved.

Some of the immediate background theory is quoted from the well-known books by C. Møller and by L. D. Landau and E. M. Lifshitz and some generally relevant ideas from the writings of F. A. E. Pirani and S. Weinberg. But the central exercise of the work seems not to have been attempted before: it is to study the consequences of as nearly as possible applying a Lorentz transformation to the Schwarzschild exterior solution, seeking

Laser tuning

from John Walker

MOST lasers have the disadvantage of operating at only a few fixed wavelengths. Dye lasers on the other hand can now be tuned over the whole visible spectrum by selecting appropriate dyes. Fine tuning is accomplished by a variety of techniques, one of which involves birefringent crystals. A recent paper (Tang and Telle, *Applied Physics Letters*, **24**, 85; 1974) reports the observation of very large tuning rates using birefringence in ammonium dihydrogen phosphate (ADP).

Earlier work needed high gain pulsed dye lasers and used rather complicated tuning elements including intracavity polarisers and gratings. Tang and Telle employed a less powerful CW dye laser, and only a single z-cut electro-optic crystal of ADP. When the z axis was parallel to the direction of propagation of the light a voltage applied across the x direction had no effect on the laser wavelength, as symmetry considerations would predict. But if the crystal were misaligned by only one degree the wavelength then unexpectedly became a very sensitive function of voltage. Tuning rates of up to $60 \text{ \AA kV}^{-1}$ were achieved, and they could be varied by changing the misalignment angle. Coarse tuning could be effected by rotating the ADP crystal. The precise explanation of the tuning effect is not known, but it seems to depend on having a crystal with very low residual birefringence in the absence of an electric field.

In any case, the method seems to be simple and useful, as Tang and Telle demonstrate in a second paper (*J. Appl. Phys.* **45**, 4503; 1974). Here they claim the first application of a laser to modulation spectroscopy, a technique which previously needed conventional light sources and spectrometers. The principle of modulation spectroscopy is quite simple. The spectrometer (or in this case laser) wavelength is caused to oscillate rapidly over a range of a few Ångströms. Using suitable electronics a signal corresponding to the first derivative of the absorption spectrum can then be extracted from the transmitted light. In practice this means that relatively weak spectral features are amplified and made visible. Tang and Telle studied the room temperature absorption spectrum of Nd^{3+} ions in a YAG crystal. They were able to resolve features which are normally seen only at liquid helium temperatures, and also detected some weak lines which are not usually seen at all.

The claimed advantages of the laser system over conventional modulation spectrometers are greater sensitivity because of the intensity of the laser light, and much better resolution. As a straightforward tunable laser the line width can be reduced to less than a tenth of an Ångström. The voltage tunability ensures very fine and reproducible wavelength selection. This versatile tuning system will undoubtedly find many applications.

thuswise to obtain a metric that might be interpreted as that of a mass in free (unaccelerated) motion. The procedure is to express the Schwarzschild solution in terms of the usual time coordinate and Cartesian-like spatial coordinates, and simply to apply a Lorentz transformation to these. Since the space-time tends to that of special relativity at large distances from the mass, the transformation has asymptotically its usual significance. The spatial coordinate system is not unique, but Salzman seeks to treat the passage from one to another admissible system as being analogous to a gauge transformation in electromagnetism. The two cases he treats in detail are "Schwarzschild coordinates" and "isotropic coordinates".

Except asymptotically, the transformation has none of the precise significance of the Lorentz transformation in special relativity. Of course, it may be qualitatively suggestive. One feature Salzman discusses that at first sight offers intri-

guing possibilities is a generalised Schwarzschild surface defined by the vanishing of the leading component (coefficient of the dt^2 term) of the metric tensor. In the coordinates used, however, this tensor is not in diagonal form, and so the vanishing of this component does not in general denote a singularity and therefore need not have any particular physical significance.

As regards the behaviour of a test particle in the field of a freely moving Schwarzschild mass, all the physical properties are derivable using any admissible coordinate system whatever. Since one is interested in only the relative motion of the particle and the mass, by far the most convenient system is the original Schwarzschild one where the metric takes the familiar form:

$$ds^2 = (1 - 2M/r)dt^2 - (1 - 2M/r)^{-1} dr^2 - r^2 d\Theta^2 - r^2 \sin^2 \Theta d\Phi^2$$

with $c=G=1$.

One relevant matter is the case of

a test particle of proper mass m_0 moving radially inwards. It is easy to show that the local velocity v along the trajectory is related to the velocity V at infinity by $(1-v^2)/(1-V^2)=(1-2M/r)$. The local rate of change of momentum is then found to be $m_0 M(1-v^2)^{-1/2}(1-2M/r)^{-1/2}$ which may be interpreted as the gravitational attraction. This agrees with the classical value in the appropriate limit. One may interpret $M(1-v^2)^{-1/2}$ as the relative mass of the body as seen by the infalling test particle. The fact that this plays the part of the gravitational mass is one of the results got in a different way by Salzman. In most applications $2M/r \ll 1$, and we may think of r as nearly enough the classical distance. But the presence of the factor $(1-2M/r)^{-1/2}$ does recall the role of the Schwarzschild surface. So as far as this simple case is concerned, it checks that there is no change in the absolute position of this surface as it affects a moving test particle. Naturally, an observer on that particle may describe the surface in various ways depending upon what observations he makes of events in its vicinity.

The simple case might suggest that there is never any need to treat a Schwarzschild mass save with the aid of a standard description of the metric. But this is obviously not the case if we have to treat the interaction of two such masses. And this is where Salzman's work may provide a valuable start. In the first place, it supplies at any rate an approximation to the required field. In the second place, Salzman does indeed obtain by formulating a suitable Lagrangian an approximation to the behaviour of interacting masses. This Lagrangian is closely similar to one formulated by Landau and Lifshitz, which may be taken to confirm the usefulness of Salzman's metric. Incidentally, he concludes that "two-body effects can be important even in the case of a 'test' mass". Also he compares his Lagrangian with that for an analogous electromagnetic problem and pursues somewhat his analogy of gauge functions, but here the physical significance is scarcely clear. At any rate, Salzman has opened up a field in classical general relativity that will undoubtedly be further explored.

Puberty

from our Steroid Biochemistry Correspondent

THE mechanisms responsible for the increased secretion of androgens at the time of puberty are incompletely understood. The use of techniques for taking frequent samples of blood, the development of simple, sensitive and specific assay methods and the polygraphic recording of the stage of sleep has made possible an investigation of hormone secretion throughout a

24 h sleep-wake cycle. These techniques have shown that some hormones, for example growth hormone, luteinising hormone (LH) and follicle stimulating hormone (FSH), are secreted episodically and that their secretion may sometimes be related to the onset of a specific stage of sleep, particularly in the pubertal state. An increase of LH secretion, which may be important in initiating androgen secretion and puberty, occurring synchronously with onset of sleep was demonstrated in pubertal, and sometimes in later pre-pubertal, boys.

In a recent study (Boyar *et al.*, *J. clin. Invest.*, **54**, 609-618, 1974) plasma LH and testosterone concentrations were measured at 20 minute intervals throughout a 24 h period in six normal pubertal boys. In all six boys there was an increase in LH concentrations with the onset of stage 4 sleep. LH was secreted episodically and although plasma testosterone concentrations did not show such marked variations, every major secretory episode of LH was followed about 20 minutes later by an increase in the plasma testosterone concentrations. Major secretory episodes of LH could occur at times when plasma testosterone concentration was increasing and close to the maximum concentration reached during the 24 hour period, whereas during the day, although testosterone concentrations decreased, no further major episodes of LH secretions were detected. In mid-puberty similar but smaller changes were observed.

To show that the increased androgen secretion was dependent upon sleep-associated secretion of LH, subjects were studied in which sustained sleep was delayed for 3 hours. Under these conditions the first LH secretory episode again coincided with the first sustained period of stage 4 sleep and was followed by an increase in testosterone secretion. Acute sleep-wake reversal studies also confirmed this relationship: concentrations of testosterone were significantly higher during the periods asleep than during the periods awake. In contrast to these results there was no consistent increase in LH secretion with the onset of sleep in sexually mature young men and testosterone secretion occurred equally throughout the 24 hour period rather than being highest during the sleep period.

The secretion of trophic hormones such as LH from the pituitary is often regarded as being controlled by a simple negative feedback mechanism; a high secretion of trophic hormone stimulates secretion of the hormone from the target gland and this increase in turn inhibits secretion of the trophic hormone. This feedback control system seems to be operating in sexually mature young men but such a control

system does not seem to explain the changes observed during sleep at puberty when secretory episodes were often maximal at times when plasma testosterone concentrations were high. These observations suggest that at puberty the central nervous system exerts a regulatory influence which is more important than the classical feedback mechanism in controlling the secretion of LH and, indirectly androgens, at puberty. The mechanisms involved in initiating hormone secretion at this time remain to be unravelled.

Binding of streptomycin to ribosomes

from a Correspondent

STREPTOMYCIN is a well-known agent for precipitating nucleic acids. In addition to this general property, the drug is also a specific inhibitor of protein synthesis, and the inhibition is associated with the smaller (30S) ribosomal sub-particle. In *Escherichia coli*, it is known that mutations in protein S12 can confer resistance to or dependence on the drug (Ozaki, Mizushima and Nomura, *Nature*, **222**, 333-339; 1969; Birge and Kurland, *Science*, **166**, 1282-1284; 1969); and that these effects can be suppressed by mutations in proteins S4 and S5 (see for review Garrett and Wittmann, *Adv. Prot. Chem.*, **27**, 277-347; 1973). The question therefore arises as to whether streptomycin binds to the nucleic acid or protein moiety of the 30S ribosome, and this is a problem which has given rise to some controversy.

Gorini and his co-workers believe that in the ribosome streptomycin binds specifically to the 16S RNA. In their latest experiments (Garvin, Biswas and Gorini, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3814-3818; 1974) they have compared the binding of streptomycin (SM) with that of dihydro-streptomycin (H_2SM), an analogue which can be conveniently labelled with tritium. Both drugs seem to have identical binding sites on isolated 16S RNA, as demonstrated by chasing experiments, and both induced quantitatively identical 'misreading' effects, when tested with 30S particles from various different streptomycin resistant strains of *E. coli*, which bind varying low amounts of streptomycin as compared to the wild type. But the binding affinity of H_2SM is lower than that of SM, since the former but not the latter could be removed from 16S RNA or 30S sub-units by simple dialysis. This effect enabled the authors to make a very interesting experiment: 30S sub-particles from wild type or streptomycin

resistant strains were exposed to H₂SM, then the drug was removed by dialysis and the sub-particle tested in the misreading system. Surprisingly, the sub-particle showed the same extent of misreading as a control sample, which had H₂SM added back during the misreading test. Thus, streptomycin is capable of inducing a new stable conformation of the ribosome, which persists after removal of the drug.

In spite of these interesting findings, the evidence that the SM or H₂SM bind specifically to the RNA moiety of the 30S particle is still not very convincing. SM binds to 16S RNA and 30S sub-particles equally well (two moles per particle), but in an earlier publication (Biswas and Gorini, *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2141-2144; 1972) a significant binding to 50S particles and 23S RNA was also found under these conditions (0.7 and 1.3 moles respectively). Further, since the binding was measured after exhaustive dialysis rather than under saturating conditions, the number of moles bound are not necessarily saturation values. Garvin *et al.* showed that SM prebound to 16S RNA could prevent the assembly of proteins under reconstitution conditions when proteins from *E. coli* B were used, whereas it had no effect when proteins from *E. coli* K were used. In the latter case, the SM remaining bound was reduced from 2 moles to 0.6 to 0.7 moles. It cannot be excluded (as the authors themselves suggest in a slightly different context) that this is due to a specific protein-mediated dislodgement of the SM from the RNA followed by rebinding to the 30S particle.

The case for binding of streptomycin to the protein moiety of the 30S particle has been put by Schreiner and Nierhaus (*J. molec. Biol.*, **81**, 71-82; 1973). These authors distinguished two types of H₂SM binding. "Type A" (high affinity) was exhibited by 30S particles, and "Type B" (low affinity) by 50S sub-particles, and 16S or 23S RNA. Type B binding could be suppressed by high concentrations of ammonium chloride, whereas the Type A binding was unaffected, and was always considerably higher than the Type B binding. A Scatchard plot revealed that at high ammonium chloride concentration the 30S particles bound 0.8 moles of H₂SM per particle (a figure which is in close agreement with the residual binding mentioned above). Binding to 30S particles could be reduced to Type B levels by removing some of the proteins with high salt, and Type A binding could then be restored by adding back the proteins in the cold. Analysis of the individual proteins showed that S3 and S5 were the most effective in this respect. But although these experiments show a direct in-

volvement of these proteins in streptomycin binding to the ribosome, they cannot be taken as proof that the drug binds directly to the protein moiety.

So, the question of where streptomycin binds remains unresolved. At the moment the evidence seems to favour the view that the proteins are more important than the RNA, but it may well turn out that, in the complex environment of the ribosome, both nucleotide and amino acid residues are involved.

Aquatic biological pollution in Florida

from a Correspondent

OF all the ways in which man has affected the flora and fauna of the world one of the most profound has been by introducing exotic species wherever human colonies have been established. In the last century acclimatisation societies flourished with the object of introducing "beneficial" animals to various parts of the world, often with severe consequences to the native biota. Today, introductions still continue but are more often due to accidental release than deliberate introduction. A recently published study by Courtenay, Sahlman, Miley, and Herrera (*Biol. Conservation*, **6**(4), 292-302; 1974) of the exotic fishes in fresh and brackish water in Florida illustrates most graphically the extent of biological pollution in this state which now contains 38 species of exotic fishes.

Thanks to its mild climate and the supply of even-temperature water from wells, Florida is the major centre in the United States for the aquarium fish industry. Some 250 fish farms exist in the state, producing nearly 80% of the pet fish for the United States. Exotic species are often unintentionally released through unprotected effluent channels, or in times of flooding. Others are dumped into open waterways when holding pools are cleared for the reception of new stock. Courtenay and his colleagues made 62 collections of fishes in central and southern Florida between July 1970 and July 1972. Thirty-eight exotic species and several hybrids between these species were found; of these 20 species and five hybrids were found to be established as breeding populations.

Not all the established exotics came directly from fish farms: some are due to release of unwanted fishes by aquarists, research workers, and to stocking by angling interests. The spread of the pike killifish (*Belonesox belizanus*) is an example. In 1957

fifty specimens were released after a medical research project was terminated; it now occurs over some 160 square miles of Dade County, and in places makes up 20% of the fish biomass, no mean feat for a slender fish of 20 cm maximum length! Others seem to be less successful—two white piranhas (*Serrasalmus rhombeus*) were caught in an (abandoned!) swimming pool in South Miami, the survivors of several after an unusually cold winter.

The walking catfish (*Clarias batrachus*) has spread rapidly and continuously since its first escape in the mid-1960s. It is an undemanding fish, capable of withstanding almost deoxygenated conditions and moderate salinity; it is also able to tolerate dessication, as well as migrating overland during rainy periods. In the dry season catfish tend to aggregate in numbers in small ponds and kill most of the animals in the pond in a few weeks.

At least eight species of cichlid fishes have become established, while others have been found. One of them, the South American black acara (*Cichlasoma bimaculatum*) is the most widely distributed exotic fish in southern Florida, and is the only exotic in the Everglades National Park. Courtenay *et al.* have found that in the Fort Lauderdale area it is the dominant fish, comprising 64% of the total fish biomass. In one canal it formed 80%, but elsewhere its contribution to the fish biomass ranged from 5 to 30%. An African cichlid, the blue tilapia (*Tilapia aurea*) is now found over the greater part of northern Florida. This cichlid was claimed by the news media to be an excellent food and game fish, and was quickly spread by fishermen to other areas from its original site of introduction dating from 1961. Unfortunately it was found to be practically valueless as a game fish, and is only now being evaluated as a food fish. It has proved to be an extremely effective competitor for the native fishes, and in many eutrophic lakes now dominates the fauna.

Courtenay and his colleagues document these and the remaining exotic species in detail, but have little to say on the effects of this biological pollution on the native fauna. Undoubtedly it has been considerable, and possibly the full effects have not yet been established. They do, however, propose some remedies to stem the flood of new exotics, most particularly in drawing attention to the state statutes which already forbid the release of non-native species. Enforcement of such statutes from the outset would have prevented much of this pollution and it is greatly to be hoped that action now can contain the problem to its present, already serious limits.

review article

Leaf protein: a beneficiary of tribulation

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In some cases, the protein content of the discarded haulm of crop plants may be as great as that of the harvested crop. After two false starts at the time of the Second World War, research on exploiting this and other sources of leaf protein may now be near to acceptance.

THERE are many diverse reasons for studying the composition of leaves and preparations made from them. The extent to which preparations are refined before study is equally diverse. Research on chemical taxonomy, or the processes involved in virus multiplication, calls for the isolation of leaf components in the greatest attainable state of purity; research on the nutritive value of fodders or green vegetables calls for no more fractionation than is involved in selective browsing, or trimming in the kitchen. Research on the use of bulk preparations of extracted leaf protein as a food for man and other non-ruminants is in an intermediate position. There is no need to separate innocuous components from leaf protein—there are indeed obvious advantages in retaining components such as β carotene—but the merits of leaf protein cannot be reliably deduced from analyses of the whole leaf because protein extraction is always incomplete and it must be assumed that there are differences between the group of proteins that remains unextracted and the group that is separated as leaf protein.

The history of research on leaf protein has been published elsewhere^{1,2} and will only be summarised here. Rouelle made it in 1773, Ereky suggested its use as food in 1925 and Slade repeated the suggestion in 1937. After the outbreak of war in 1939, several groups of scientists met to discuss how scientific knowledge and skill could best be used. The conclusions were communicated to those charged with care for our welfare but no attention was paid to the suggestion that leaf protein could help to alleviate protein shortage if there were a blockade. The mental climate changed after the 'fall of France' and support for the project began. In the second half of 1940, and in 1941, large-scale equipment of many types was examined to see whether any existing machinery was suitable for extracting juice from leaves.

All types had defects. Understandably, this work stopped with the arrival of 'Lend-Lease' food.

Liberating the juice

Tribulations at the end of the war, for example the ending of 'Lend-Lease' and the growing awareness that much of the world was chronically undernourished, reawakened an interest in leaf protein that persisted for 4 or 5 yr. Interest then waned and work was languishing until we received grants from the Rockefeller Foundation and later from the Wolfson Foundation. With these grants, and on the basis of the wartime studies of machinery, several extraction units were made. The design depended on the idea that liberating juice from leaf cells differs qualitatively from expressing it from leaf pulp. Separate units were therefore used for the two processes. We concluded that rubbing is more important than fine subdivision, that rubbing leaf against leaf is as effective as rubbing leaf against steel, that the layer of pulp from which juice is being pressed should be less than 1 cm thick, that pressure should be maintained for at least 7 s to allow juice to flow away from the pressed fibre, and that there is little advantage in applying a pressure greater than 4 kg cm⁻². Units conforming to these principles have been made that handle up to 5 ton of crop per hour. The pulpers run at 1,000–3,000 r.p.m. and so waste energy in creating wind, but the presses are efficient.

The advantages of liberating and expressing juice in one operation are obvious. At various times and places rollers and screw expellers have been used with partial success, as pure pressure extracts little protein from leaves. Rollers extract protein because there is some slip, which rubs leaf against leaf,

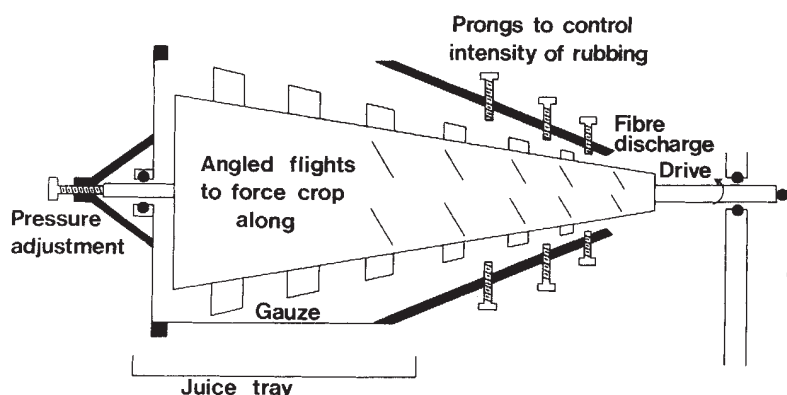


Fig. 1 Screw expeller for juice extraction.

as the charge enters the 'nip', but pressure is too brief to allow juice to run away and much of it is readsorbed by the fibre. The scroll of a screw expeller rubs across the charge; this liberates juice but wastes energy in useless friction. A remodelled screw expeller in which the scroll is broken into many paddles, so that the charge is constantly rearranged and stirred as well as being pressed, should use more of the input energy to rub and tear, rather than heat, the crop. Figure 1 illustrates the principle of what is proposed. This arrangement has the additional advantage that juice is not expressed under pressure but flows by gravity through a gauze. When juice is forced through a packed mass of leaf fibre, much of the protein in organelles and their fragments is retained, and some of the finer fibre particles contaminate the juice. For quantitative agronomic studies on the yields of leaf protein attainable from different crops and systems of husbandry it will probably be better to pulp and press in separate units because it is then possible to standardise conditions more completely².

Food or fodder

Research on large-scale production of leaf protein started as a means for making human food for use in Britain and it was restarted after the war, partly for the same reason but more because it seemed likely that in the wet tropics, the yield of leaf protein would be greater than the yield of other protein concentrates. At that time the need for protein concentrates was universally agreed and the Food and Agriculture Organisation took an active interest in concentrates from such sources as oil-seeds and fish. FAO was unsympathetic towards work on leaf protein and in several publications asserted that it was unpalatable and much too expensive. Those having practical experience with properly made leaf protein knew that it was palatable—especially in countries where curry or powdered dried leaves are traditionally used as relishes. Calculations based on experience in the grass drying industry suggested that leaf protein would be as cheap as any other concentrate that was not a by-product. With FAO we were presumably up against the fundamental objections succinctly stated by Professor Mary Douglas³ "There wouldn't be time", "We couldn't afford it", "God doesn't like that sort of thing" and the ultimate "It's against Nature". The International Biological Programme was more open-minded. Research on leaf protein formed part of the national programmes of India, New Zealand, Nigeria, Sweden and the United Kingdom, and under its auspices much valuable research has been done.

Although agronomic information, and information about the effect of processing conditions on the properties of leaf protein was accumulated, there was little commercial interest. Several patents have been taken out; many of them restate facts familiar since 1773 and none cover points that are essential for commercial production. There is, or has been, commercial interest in Brazil, Eire, France, Hungary, Japan, New Zealand, Sweden, UK and USA. The actual present status of these projects is uncertain, and some of them may have failed because of the use of unsuitable equipment.

When large-scale research started it was obvious⁴ that the fibre from which juice had been expressed could be dried more economically than the forage from which it had been made. To get a ton of dry matter from a forage, 5 to 10 ton of water must be evaporated, whereas it is only necessary to evaporate 2 or 3 ton from the fibre. The product contains less nitrogen than the corresponding dried fodder but it is less lignified than a fodder with the same nitrogen content because it would have been made from a less mature crop. Furthermore, because soluble materials are largely removed from the fibre, species of leaf could be used as fodders which in the original state, are unpalatable, or even toxic to ruminants. These points were made in a note circulated by the Agricultural Research Council in 1951 and they have been stressed repeatedly (see, for example,

ref. 5) since then. Even if a crop is being ensiled rather than dried, extraction of juice may be prudent because it is all extracted at once, and can therefore be collected and used, instead of seeping out slowly as a polluting effluent. These advantages of fodder fractionation received little attention until the tribulations resulting from the increased price of oil focused attention on wasteful processes in general. Now, most interest is being shown in fodder fractionation as a means for conserving fodder economically; leaf protein is regarded as a by-product for feeding to pigs and poultry. There is active research at the National Institute for Research in Dairying (Shinfield), the Rowett Research Institute (Aberdeen), and the Agricultural Institute (Dublin). In some ways it is a pity that the objective has changed in this way, but the research that is being done suggests that FAO was wrong in arguing that leaf protein would be very expensive, and the experience gained will in time be used in the production of human food. Unfortunately, leaf protein will by that time have gained the stigma of being fodder and this will slightly impede acceptance by people.

Sources of leaf

Conventional crops, harvested at an immature stage, are the sources of all bulk preparations of leaf protein. At Rothamsted we prefer the cereals and clovers, while in New Zealand and USA lucerne is preferred. Batches of protein weighing a few kg have been made in Britain, India and Sweden from 20 or 30 other species—many of them normally classed as weeds. Even if species conventionally grown as sources of seed are used, it may well be that the varieties rejected by plant breeders would be better for our purpose than those that give a good yield of seed. At Rothamsted we can get 2 ton of dry extracted protein (calculated at 16% N) annually per hectare and expect to be able to get 3 ton. In India the yield is already 3 ton and it should be possible to get 5 ton. It is not certain that such large yields should be aimed at as they call for a level of fertiliser use that may not be economically sensible.

Leaves that are a by-product from a conventional crop are the ideal source of leaf protein. Potato haulm has been examined in some detail at Rothamsted; the yield of extractable protein would probably be about 600 kg ha⁻¹ at the end of July but only 100 kg ha⁻¹ in mid-September. Prudent farmers destroy the haulm at the beginning of September as a safeguard against blight; prudence would probably be more widespread if farmers realised that something valuable could be made from haulm. Bearing this in mind, and adding in the haulm from seed and early potatoes, about 60,000 ton of protein is now being wasted in Britain by destroying rather than harvesting haulm. A larger amount of protein is wasted in sugar beet tops but that source has been studied less systematically. With varieties used now, more protein can be extracted from the haulm of peas harvested for freezing and canning than is present in peas themselves. Outside leaves and other forms of waste from the vegetable industry are another large but uncertain source—uncertain because vegetables found to be surplus after reaching the shops have often deteriorated too much to be useful, and outside leaves are often damaged and would be troublesome to collect in the field. The potentialities of this source should be surveyed. One point about vegetable leaves must be stated clearly: there is no advantage in extracting protein from material that could be eaten. Few communities eat as much green-stuff as is desirable and leaf protein is more conveniently eaten in the leaf than after extraction.

Although many species now classed as weeds will be grown deliberately as sources of leaf protein, the mixed unfertilised vegetation on waste land is not a probable source. If a harvesting implement could be used on land, the land should be re-sown and properly fertilised. Water weeds are different. They are usually well fertilised and the growth in an area tends to be dominated by a single species. According to some estimates,

the annual cost of trying to control water weeds with herbicides or by mechanical destruction is £500 million: hardly anything is being spent on trying to use them. Enough has, however, been done to show² that leaf protein can be extracted satisfactorily from some species.

Protein quality

Chloroplasts, their fragments, and other organelles begin to coagulate when leaf juice is heated to about 50° C, coagulation is complete at 70° C. By separating a low-temperature coagulum, and then heating the fluid further, a green fraction containing 7–9% N and a fawn fraction containing 9–12% N can be made from most species that extract satisfactorily. This may sometimes be a useful procedure, but it introduces complexities into what is otherwise an extremely simple process; the low-temperature coagulum is too soft to filter off easily. When juice is heated suddenly to 70° C a dense, easily filtered curd is formed. Sudden heating inactivates chlorophyllase⁶ and so prevents the formation of phaeophorbide; this was formed to a sufficient extent in slowly heated juice to photosensitise animals fed on the product⁷. The press-cake, after washing to remove soluble leaf components, should have the composition shown in Table 1. The fats are highly unsaturated. This causes trouble during preservation and storage because unsaturated fat combines with

species differences are greater than differences between preparations made from the same species harvested at different ages or in different conditions of husbandry. This uniformity is not unexpected because what is loosely called leaf protein is a mixture of many different proteins and the same amino acid deficit or excess is not likely to characterise all of them. Amino acid analysis suggests that properly made leaf protein will have good nutritional value. This expectation was borne out by experiments on the growth rate of chickens, pigs and rats and by brief experiments on the amount of nitrogen retained by infants fed on milk alone and on mixtures of milk and leaf protein. These results have been confirmed² and extended by a six month experiment in which school children, on a diet consisting predominantly of ragi (*Eleusine coracana*), were given supplements containing 10 g of protein per day in the form of lucerne leaf protein, or sesame seed (*Sesamum indicum*). The leaf protein gave better results, assessed from height, weight and haemoglobin content, than sesame although the protein in the latter is regarded as one of the best seed proteins.

Acceptability

Leaf protein should not be regarded as a medicine and eaten in the form of pills. It should rather become one of the many components from which a mixed diet is made up. But people do not, as a rule, think of their diets in terms of so much energy, so much protein and so on per day. They eat foods with familiar appearance for traditional reasons. Methods must, therefore, be found for presenting any novel foodstuff in an acceptable way. Fresh leaf protein is a slightly acid cake containing 60% water and with the texture of cheese. It can be pickled, salted or dried. It will be most readily accepted by communities that already make regular use of green vegetables and, as already mentioned, increased use of leafy vegetables is the most sensible way of getting leaf protein eaten. The next step depends on local culinary habits. As a result of present-day cultural interpenetration, the food shown in advertisements in publications coming from wealthy countries are gaining prestige, while the traditional stews and gruels of much of the world, into which many novel foods, such as leaf protein, would fit easily, are losing it. This is understandable but unfortunate because the foods of the wealthy are getting scarce so that expectations that are not likely to be fulfilled are being fostered. It is likely therefore that novel foods, on which work was originally undertaken so that they could relieve malnutrition in developing countries, will not be acceptable there until they are manifestly used by the wealthy. Acceptance will be impeded if they are first used as welfare foods or foods for the unfortunate.

In some parts of the world the need for protein could be most economically satisfied by growing yeast or some other micro-organism on molasses or oil. In others the best source may be a seed such as groundnut or soya. But in rural tropical regions where rainfall is regular, or even excessive, leaves are the most productive source of protein: these regions are at present the worst fed parts of the world. Tribulation has thrice energised research on the production of leaf protein and on its use as food for people and other non-ruminants. Research may now have progressed so far that the logical case for production of leaf protein is strong enough to prevail against habit and preconception. It is possible, however, that a fourth jolt will be needed. If economists and other prophets are to be believed, no scarcity of tribulation is imminent.

protein in a manner that makes it less digestible and keeps some of the constituent amino acids from being effectively metabolised. These fats also undergo 'oxidative rancidity.' This enhances the value of products such as tea and smoked fish but it is usually regarded as detrimental in other foods. Dry leaf protein that has been stored in air, slowly develops a characteristic flavour. As with tea and smoked fish, one learns to like it but, in the early phases of popularisation, the less flavour it has the better.

Protein does not extract well from leaves rich in tannin or phenolic compounds. Even small amounts of these substances, though they may not prevent extraction, combine with the protein as unsaturated fats do. Differences in the extent to which these complexes were formed during extraction or storage, probably explain the differences in nutritional value that have sometimes been observed between samples of protein with similar amino acid compositions. It will probably not be possible, in the course of bulk production, to prevent combination between protein and phenolic compounds. That form of damage could be circumvented by selecting species and stages of growth that ensure that the leaves are relatively free from phenols.

Some people dislike the green colour of leaf protein made by the simple method outlined. Extracting the colour with a solvent is easy—but it adds to the cost, and the useful β carotene and unsaturated fats would then be lost. Furthermore, leaf protein production has not only the merit of yielding an unprecedentedly large amount of edible protein, it is also so simple that it could be a small-scale industry in any community able to manage a tractor. It will lose that merit if solvent extraction is insisted on.

All leaf protein preparations for which there are reliable figures have similar amino acid compositions⁸. It is unlikely that

Table 1 Composition of leaf protein as usually prepared

True protein	60–70%
Lipid	20–30%
Starch	5–10%
β Carotene	1–2 mg per g
Fibre	<2%
Water soluble compounds	<1%
Ash	<3%
Acid insoluble ash	<1%

¹ Pirie, N. W., *Science*, **152**, 1701 (1966).

² Pirie, N. W. (ed.), 'Leaf protein: its agronomy, preparation, quality and use'. IBP Handbook 20 (Blackwell, Oxford, 1971).

³ Douglas, M., in *Ecology in Theory and Practice* (edit. by Benthall, J.), 129 (Viking Press, New York, 1973).

⁴ Pirie, N. W., *Chem. Ind.*, **61**, 45 (1942); *Nature*, **149**, 251 (1942).

⁵ Pirie, N. W., *Fertil. Feed. Stuffs J.*, **63**, 119 (1966).

⁶ Arkcoll, D. B., and Holden, M., *J. Sci. Fd. Agric.*, **24**, 1217 (1973).

⁷ Lohrey, E., Tapper, B., and Hove, E. L., *Br. J. Nutr.*, **31**, 159 (1974).

⁸ Byers, M., *J. Sci. Fd. Agric.*, **22**, 242 (1971).

articles

Earthquake simulation by nuclear explosions

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A country wishing to evade a comprehensive test ban treaty could disguise a clandestine nuclear test as a natural earthquake by detonating a sequence of explosions, one of which would be caused by the device of interest.

ANY discussion of a treaty prohibiting underground nuclear weapon tests (that is, a comprehensive test ban treaty, CTBT) must address the crucial issue of how to monitor whether or not the treaty is being observed. Much of the current literature ignores the possibility of schemes to conceal or disguise clandestine testing, so here we describe the reasons for believing that illicit nuclear tests, with yields up to at least 100 kilotons (kton) are feasible. Further, such tests can be carried out with little likelihood of discovery.

Seismic monitoring

The seismological literature describes numerous techniques that are directed towards identifying the source of a seismic signal as either an explosion or an earthquake. In general, they are based on features of the signals that can be explained in terms of the known gross differences in physical source mechanisms between earthquakes and single explosions. Elements of confusion enter into the use of all known discriminants, however, because the real Earth is far from an idealised, homogeneous body. Interfaces within the Earth, for instance, convert some of the compressive (P) wave energy into shear (S) waves, this tending to remove a deficiency that would otherwise be a tell-tale characteristic of explosion signals. Rayleigh and Love waves transmitted along the Earth's surface are nonetheless generally stronger in the radiation from an earthquake than in that from an explosion, but the quantitative differences are not dependable; and even though the first motion of the recorded signal from the two source types would, in principle, be distinctly different, natural background noise in general obscures it.

The salient differences between earthquake and explosion are:

- The first motion of earthquake seismic waves is rarefactional in some directions (sometimes only along rays confined to 100–200 km from the source). Seismic-wave first motions from an explosion, on the other hand, are everywhere compressional.
- P-wave trains from an earthquake are often extended in time whereas those from an explosion tend to be more compact. This results in greater P-wave 'complexity' for some earthquakes than for explosions¹.
- Seismograms from deep earthquakes frequently exhibit a phase (the pP phase) caused by reflection from the Earth's surface above the earthquake. This follows the initial downward-directed P phase by a time interval proportional to the focal depth. This interval also increases with the distance to

the detection station, an effect called 'stepout'. Explosions are seldom deep enough to exhibit a clearly separated pP phase or stepout.

- The surface waves generated by an earthquake carry relatively more energy than do those generated by an explosion of the same body-wave magnitude. Thus the surface-wave magnitude, M_s , for an earthquake is usually greater than that associated with an explosion of the same body-wave magnitude, m_b (ref. 2).

Seismic evasion

We have attempted to simulate the seismic signal of an earthquake by superposing eight seismograms of a single explosion. These were identical in shape, being simply scaled versions of the explosion seismogram actually recorded at a single monitoring station. Each seismogram was delayed in time relative to the first and scaled in amplitude to correspond to that of an explosion of selected yield. This procedure was carried out for each of three different monitoring stations.

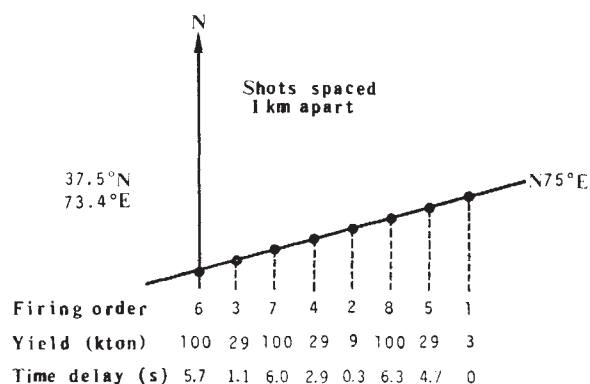


Fig. 1 Geometry, firing order, yields and time delays of the eight explosions selected to simulate an earthquake.

Figure 1 shows the proposed spatial and temporal separations of the shot array. In order to take travel times realistically into account we postulated a specific test site, at 37.5°N and 73.4°E in southern Tadzhik, SSR, coinciding with the epicentre of the earthquake we used for comparison. (Lack of a suitable comparison earthquake record prevented us from choosing a more realistic site, such as the area around Lake Baikal.) The azimuth, N75°E, was chosen arbitrarily and because detonation under a river would present many advantages, such as masking surface disturbances both before and after detonation, we chose a linear array of explosions.

We formed the composite, synthetic seismograms at the

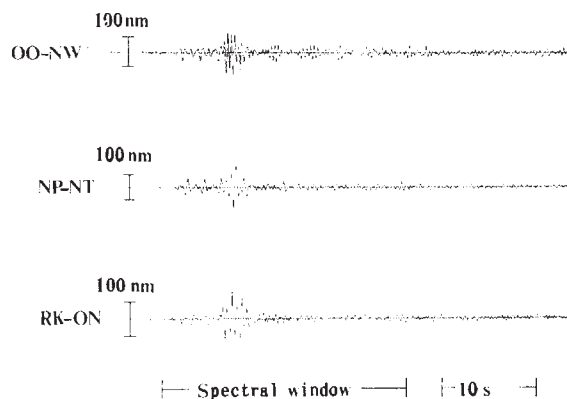


Fig. 2 Composite, short-period, synthetic seismograms for the shot array of Fig. 1 as they would be recorded at three seismic stations.

three seismic stations: Mould Bay, Northwest Territory (NP-NT); Oslo, Norway (OO-NW); and Red Lake, Ontario (RK-ON). The operations involved were

$$P(t) = \sum_{i=1}^8 p(W_i, t - t_i - T_i) + n(t)$$

$$p(W_i, t) = (W_i/100)^{0.9} p(t)$$

where $P(t)$ is the composite, synthetic seismogram at a station and $p(t)$ is the filtered seismogram of a Semipalatinsk (Soviet nuclear test site) event as recorded at that same station. The other quantities appearing in the equations are $n(t)$, a micro-seismic noise recording; T_i , the individual travel times from explosion point to detector site; and W_i and t_i , the yields and delay times specified in Fig. 1.

The United States Coast and Geodetic Survey (USCGS) reported that the explosion which generated $p(t)$ occurred on March 3, 1965 at 06:14:57 GMT. The event coordinates are 49.8°N, 78.1°E with depth constrained to the surface of the Earth. From the body-wave magnitude, m_b 5.6, we infer a yield of about 100 kton in hard rock.

The sequence in Fig. 1 was not optimised for evasion. It is presented only as an example of a sequence whose seismic record would probably be identified as that of an earthquake.

Figure 2 shows the composite, short period, synthetic seismograms for the three stations and Fig. 3 the seismograms of an earthquake that occurred at the postulated test site on February 2, 1965. The USCGS reported the earthquake origin time as 15:56:51.0 GMT and the magnitude as m_b 5.8. The two sets of seismograms are sufficiently similar that such qualitative inspection alone is unlikely to arouse an analyst's suspicions.

The following observations are based on more detailed examination of the synthetic records:

- First motions might be interpreted as rarefactional in two (OO-NW and NP-NT) of the three examples presented. Identification as an earthquake on this basis is unlikely, however, as the low signal-to-noise ratio makes the interpretation ambiguous.
- The three high yield explosions produce signals very suggestive of a pP phase. Again, the uncertainties are such that this could not be regarded as identifying the event positively as an earthquake. This aspect is not crucial, however, for many seismic regions that are suitable as testing sites produce earthquake signals lacking such a reflection phase.
- The body-wave magnitude would be about 5.1, equivalent to the magnitude of a 50-kton explosion in hard rock.
- The time duration of the composite P-wave signal is longer than that appropriate to a 100-kton explosion and its complexity is greater.

- We examined the Fourier spectra of the explosion on March 3, 1965 and the earthquake on February 2, 1965 at each of the three stations. The shot array spectra show no systematic pattern of peaks and nulls suggestive of source interference phenomena and overall comparison of the two sets of spectra does not reveal any other features indicative of the shot sequence.

- Rayleigh waves detected at long ranges, whether from an earthquake or an explosion, have periods chiefly in the 20-s band. Thus, the separate signals from a series of explosions occurring within a few seconds of one another will superpose constructively.

We illustrated the latter point by superposing (with itself) the Rayleigh wave signal from an earthquake which occurred in the Kermadec Islands on March 3, 1965. (The USCGS origin time and magnitude were 05:52:57.4 GMT and m_b 4.7, respectively.) This signal was scaled, shifted and superposed in the same way as the short period signal. The amplitude of the resultant superposition is about three times larger than that of the largest individual signal. Accordingly, the surface-wave magnitude M_s of the superposition is about 0.5 units greater than that expected from a single 100-kton explosion. We used the Rayleigh waves from an earthquake because the Rayleigh waves from the explosion on March 3, 1965 were obscured by the seismic signals from the earthquake at two of the three stations and were too weak to observe at the third. This substitution does not however, affect our argument. Rayleigh waves from earthquakes have the same periods as those from explosions and we are showing only the effects of superposition.

The net effects of modifications to body and surface-wave magnitude are shown in the $M_s:m_b$ plot, characteristic of the postulated shot array region (Fig. 4, adapted from Fig. 7 of ref. 3). The signal from a single 100-kton explosion would be expected to lie within box 1 (the width of which is intended to be representative of the spread in M_s values associated with a given m_b .) The body-wave magnitude of the superposition is reduced to 5.1 which would result in an $M_s:m_b$ value lying within box 2 provided the surface wave magnitude were that corresponding to a single 100-kton explosion. Enhancing M_s by 0.5 units (the effect of constructive interference of the surface waves) moves the point into box 3. This falls comfortably within the earthquake population.

Thus the signal, when subjected to typical discrimination analysis, appears more earthquake-like than explosion-like.

Practical considerations

We have studied the engineering, logistic, camouflage and diagnostic considerations that would be crucial to the actual conduct of such a clandestine test. Although a detailed discussion

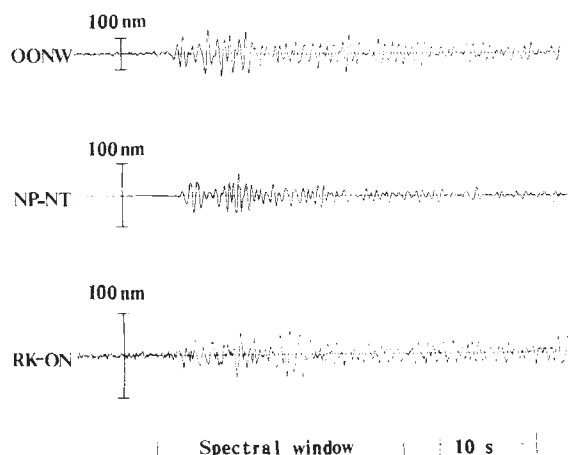


Fig. 3 Seismograms showing P waves of an earthquake near the Russia-Afghanistan-China border intersection as recorded at three seismic stations.

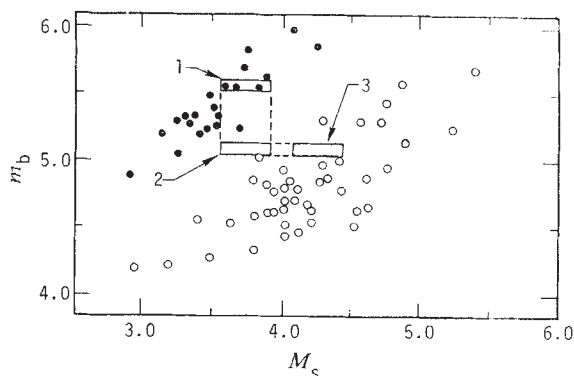


Fig. 4 Surface-wave magnitude (M_s) against body-wave magnitude (m_b) for a population of earthquakes and explosions and for the eight-shot array discussed here. All events are from a region around the assumed test site. Adapted from ref. 3.
●, Explosions; ○, earthquakes.

lies outside the scope of this article, we conclude that none of these matters would present serious problems.

Briefly summarising, camouflage might well be accomplished by detonating the explosives under a river bed. We have carried out a complete feasibility study of this unconventional operation. Including the conceptual design of a suitable drill barge and its associated drilling equipment, the development of complete pre-shot and post-shot operational plans, the development of a drilling plan, including operation of a drill-barge, design and emplacement of a canister containing both device and diagnostics and device detonation. The important result was that the whole operation could be accomplished with current technology, albeit assuming the high level of technical competence in the domain of nuclear testing to be expected of mature nuclear powers.

The operational concept was shown to evade all possible detection threats, for example:

- Increased river activity is accounted for to the local populace by initiation of a suitable construction project.
- Overhead surveillance is thwarted by carrying out drilling or other identifiable operations only at night.
- Drilling chips are discharged downstream, in deep water.
- Surface expressions are averted by detonating the explosions underwater.

We acknowledge that such an operation would call for special care and effort and would entail greater expense than non-prohibited tests. In the feasibility study alluded to we also considered these matters. We are confident that the additional difficulties resulting from the clandestine nature of the test can be overcome at acceptable levels of expense and effort. With dedication and suitable inducement a test crew of reasonable size (~100) could carry out the entire operation in about four months. This conclusion is again based on US test experience and short extrapolations from that. We estimated that costs would not exceed those of a single conventional test by more than a factor of 30. Thus, although such a test would be expensive, its cost need not deter a determined evader.

If, on the other hand, the drilling were done on land, avoidance of the visible or other detectable effects of a clandestine explosion would become a prime requirement. Potential effects include, primarily, various alterations of ground surface contours over the explosion and the release of radioactive effluents. A careful evader with test experience could predict such effects with confidence.

Surface collapse craters are common and very visible phenomena at underground nuclear explosion sites⁴. Their probability of occurrence depends on the local rock type as well as the yield and depth of the explosion. Experience in the United States shows that a burial depth (in metres) of at least 300 times the cube root of the yield (W), expressed in kton, will prevent surface cratering regardless of the rock type.

Other visible effects can include minor surface cracking related to ground disturbances and settlement, and surface expression of fault motion stimulated by the explosion. These are observed within a few kilometres of US tests, where the typical burial depth is in the range of $120W^{1/3}$ (ref. 5). Offsets of a few centimetres are common and cases of dislocations measuring a metre or more have occurred. Burial at greater than normal depth would probably, though not certainly, eliminate these effects. Gasbuggy, Rulison, and Rio Blanco, the three deep US nuclear explosions fired to test stimulation of natural gas production, produced no observed fault motion or surface cracking. Their depths of burial were all at least $400W^{1/3}$ m.

Venting of radioactive material would in all likelihood be completely absent and at most would be limited to trace seepage of gases, detectable only by sensitive survey instruments at the explosion site. Present containment techniques in use by the United States are highly successful at normal burial depths and would unquestionably assure containment at the greater depths appropriate to a clandestine explosion.

Finally, and very briefly, precautions of a practical nature which an evader would likely take are:

- Carry out the test in an area of low population density with at least moderate seismicity.
- Use the smallest possible test crew.
- Devise a cover operation to deceive the local populace and provide an excuse for whatever relocations might be necessary.
- Detonate before dawn to catch most of the local population asleep. Avoid detonation on an even hour, half hour or minute, as has been common practice in previous non-prohibited tests.
- Issue a routine earthquake announcement. The details would be misleading (but not so misleading as to arouse suspicion).
- Select highly reliable explosives to be included with the test device in the explosion sequence. (We did, however, study the effect of failure of any one of the eight shots, but it is small and does not change the conclusions presented here.)

We believe that these precautions, and others, can be followed by a determined, resourceful evader with previous field test experience and a stockpile of proven, reliable weapons.

Reassessment

We have outlined in this article a possible way of carrying out clandestine nuclear tests. The arrangement produces a seismic signal that would be identified by seismic analysts as that of an earthquake on the basis of the $M_s:m_b$ criterion. All other criteria considered are at least consistent with, or suggestive of, identification of the event as an earthquake.

We made no attempt to optimise yield, number of explosions or any other parameter, or to identify the maximum yield that could be tested. Even so, we demonstrate a substantial enhancement of yield capability over the detection threshold for an undisguised test.

Further, the maximum yield permitted by this evasion scheme would still not tell the whole story. Weapons of still higher operational yield could be developed and stockpiled if an evader were willing to risk extrapolating their expected performance from partial-yield experiments.

We are aware of intensive current work on new discriminants, for example, Love-to-Rayleigh spectra ratios, radiation patterns and newer techniques for depth determination. These newer discriminants are generally incompletely developed, but even if they were applied the superposition presented here would be classified as an earthquake according to present standards. It met the $M_s:m_b$ criterion and, as discriminants are used at present, satisfaction of just one discriminant results in identification. (This is because any requirement that more than one criterion be satisfied would preclude identification of most events.)

We believe that recent statements concerning identification of nuclear tests in the 10-kton range must be understood to

apply only to the current open mode of underground testing. If a CTBT were to emerge, the relevant seismic monitoring capability should take into account competent efforts at evasion. We suggest that such a reassessment must be factored into the proposed safeguards for a CTBT.

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The subunit structure of the eukaryotic chromosome

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New structural data have been obtained from neutron scattering studies of chromatin. The concentration-dependent meridional peak at 10–11 nm comes from the interparticle spacing of a subunit structure. Peaks at 5.5 and 3.7 nm have a different contrast behaviour to those at 11.0 and 3.7 nm showing that histones and DNA have a different spatial arrangement in the subunit. A globular model in which apolar segments of histones form the core surrounded by DNA complexed with the basic segments of histones agrees with the data.

STRONG evidence for a basic structural repeat in eukaryotic chromosomes comes from the observation of a series of rings at about 11.0, 5.5, 3.7, 2.7 and 2.2 nm in the X-ray patterns of native and reconstituted chromatins^{1–8}; they do not seem to have been observed in the X-ray pattern of DNA nor in the X-ray pattern of total histone. In 1964, Wilkins proposed a model of a uniform supercoil with a pitch of 12.0 nm and an outer diameter of 13.0 nm to explain this characteristic X-ray pattern, and in the model calculations it should be noted that only the X-ray scatter of the DNA component was considered⁶. Another, more irregular supercoil model (pitch 4.5–7.0 nm, outer diameter 8.0–12.0 nm) has been proposed⁹. An extension of the idea of the supercoil is a polyhelical model¹⁰. Several authors have proposed a globular subunit structure for chromatin^{11–15}. 'Linear arrays of spherical chromatin particles have been observed'^{11–13}, of about 7.0 nm diameter joined by threads 1.5 nm diameter. This 'particles-on-a-string' model is supported by further observations: endonuclease digestion of chromatin gives DNA pieces in integral units of about 200 base pairs^{16–18}, and nuclease digestion a basic subunit of 205 base pairs and a second degradation subunit of 170 base pairs¹⁹. It has also been postulated¹⁸ that tetramers of histones (H3 and H4), observed in histone gently dissociated from chromatin^{20–21}, associate with 200 base pairs to give a subunit of chromatin.

Clearly, additional data are required and for this reason we have used neutron techniques. Small angle neutron scatter

techniques have particular application to studies of structures of complex biological systems at low resolution^{22–25}. The very large difference between the average neutron atomic scattering factors per atom for H₂O (-0.06×10^{-12} cm) and D₂O ($+0.63 \times 10^{-12}$ cm) allows the scattering from all biological molecules to be contrast-matched. Figure 1 shows that neutron scatter from DNA is expected to be matched at about 63 D₂O% and that of

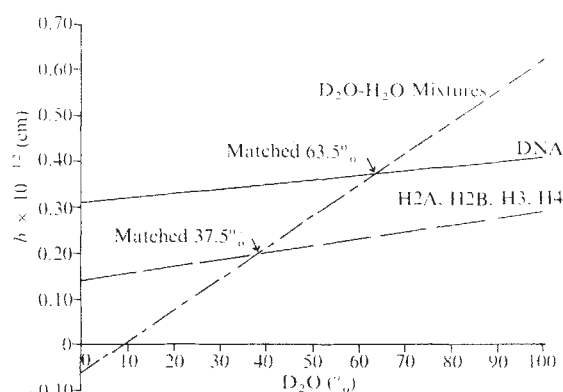


Fig. 1 Computed neutron scatter from the components of chromatin. Mean neutron atomic scattering amplitude (*b*) against percentage D₂O in the D₂O + H₂O mixture for H2B, H2A, H4 and H3 histones together, and for DNA. The plots for histone and DNA are sloping because of the exchange of labile protons with deuterium and thus is proportional to the amount of D₂O in the aqueous mixture.

histones at about 37.5% D₂O. In practice, however, using hydrated H1-depleted histone films, the matching occurs at 44% D₂O. The large difference in the proportions of D₂O required to match histones and DNA provides an opportunity to study the relative disposition of histones and DNA in chromatin.

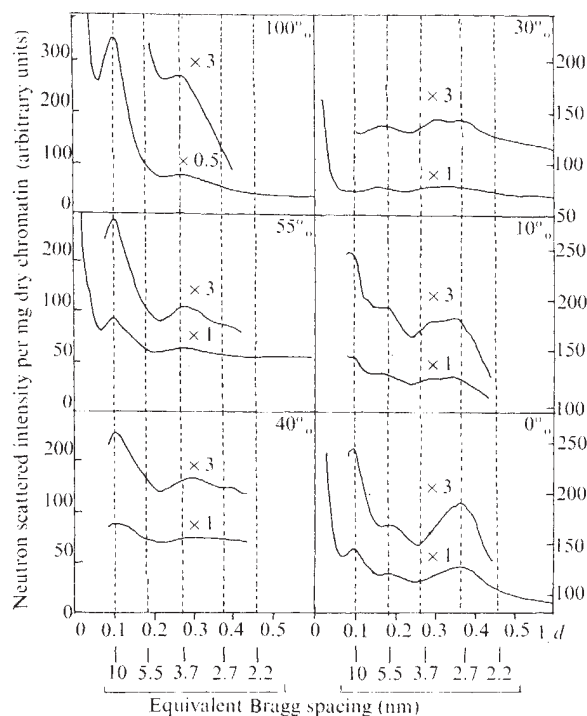


Fig. 2 Contrast variation of the radial distribution (averaged over 360°) of neutron scattered intensity for total chromatin films at 50% w/w concentration; $1/d$ is the reciprocal of the equivalent Bragg spacing (given below). The chromatin was isolated from fresh calf thymus by the method of Panyim *et al.*³⁶. Chromatin and H1-depleted chromatin³⁷ were treated with 0.5 M HCl for 2 h at 4° C, precipitated with six volumes of acetone and washed three times with acetone. After vacuum drying the histones were analysed by polyacrylamide gel electrophoresis³⁸. The protein/DNA ratios were measured by infrared spectroscopy, with suitable corrections as reported previously³⁹, which also shows any sample impurities. Experiments were carried out at 6 Å with the small angle scattering equipment (NLS)²⁴ at the ILL, Grenoble. The graphs have been redrawn on different scales, the factors by which the originals were changed being indicated on the appropriate curve. The percentage D₂O is given in each case.

Contrast variation studies in chromatin

Contrast variation experiments, using H1-depleted and total chromatin gel equilibrated with vapour from mixtures of H₂O and D₂O, provided results over a range of relative humidities (r.h.). Those for 50% w/w concentration of chromatin at 98% r.h. are shown in Fig. 2. Although the resolution is lower than for X rays, the curve which approximates to the X-ray patterns is that at 10% D₂O, which is also approximately the composition at which the solvent neutron scatter is almost zero. The effect of changing the H₂O/D₂O ratio is striking. At 100% D₂O the 10.0 nm (approximate) ring is dominant and there is a clear ring at 3.7 nm. As the D₂O content is reduced the intensity of this 10.0 nm ring weakens to become zero at 30% D₂O, after which it increases again. A plot of the square root of the intensity is linear and goes through zero at 30% D₂O. The background scatter underlying the diffraction rings also levels at the same D₂O/H₂O ratio. The 3.7 nm ring behaves similarly but retains a low intensity at 30% D₂O (Fig. 2). The 5.5 and 2.7 nm rings, however, are strongest at 0% D₂O and weaken in intensity with increasing D₂O content, until the 5.5 nm ring is no longer observable and the 2.7 nm ring is very weak. Similar results were observed with H1-depleted chromatin at about 9% w/w, except that the intensity of the lowest angle ring is reduced to zero at about 44% D₂O. At 77% w/w, the lowest angle ring is found at 9.0 nm, reaching zero intensity at 0% D₂O. The 10.0 nm ring seems to be a Bragg peak in a

region where scattering comes mainly from the protein component of the chromatin subunit. The other peaks may be part of the Fourier transform of the individual subunits, the 5.5 and 2.7 nm peaks arising from the transform of the DNA component and the 3.7 nm peak from the arrangement of proteins in the subunit. The background scatter also seems to come from the protein component.

One of the chromatin films used was stretched and the neutron scatter pattern recorded after equilibration in 98% r.h., 100% D₂O. Figure 3 shows that the 10.5 nm ring has a meridional orientation and the background scatter a strong equatorial orientation. With other camera settings, this equatorial scatter extended to about 4.0 nm equivalent spacing.

Concentration dependence in H₂O and D₂O

There is a marked concentration dependence of the intensities of the low angle X-ray rings of chromatin^{1,2,6}. With increasing concentration the ring at about 11.0 nm weakens and disappears, whereas the higher angle rings in sequence initially increase in intensity then weaken and disappear. Finally, in the dry state two rings are found at about 8.0 and 4.0 nm. Attempts have been made to explain this behaviour by changes in the packing of regular supercoils⁵. With neutrons a similar behaviour is observed for chromatin in H₂O (Fig. 4). The neutron ring at about 10.5 nm weakens with increasing concentration reaching zero intensity at 77% w/w and re-appearing at 8.0 nm at the highest concentrations. At 77% w/w, the background scatter underlying the diffraction rings also levels in a manner similar to the contrast matching against D₂O/H₂O ratios described above. Again, the resolution is lower than for X rays; the intensities in the other rings seem to move to higher angles with increasing concentration. In D₂O, there is a markedly different concentration dependence of the intensities of the diffraction rings (Fig. 5), crucial to our understanding of the chromatin diffraction patterns. In contrast to the behaviour in H₂O the lowest angle ring does not go through zero intensity with increasing concentration. Because of the similarity of H₂O and D₂O this difference, and therefore the concentration dependence of the intensities of both X-ray and neutron rings in H₂O, cannot be ascribed to structural changes. With increasing concentration, however, the peak moves from about 10.5 nm to 8.4 nm, consistent with closer packing of the chromatin subunits. Throughout the changes in D₂O, the 3.7 nm peak is constant, indicating a different origin to the variable 11.0 nm ring.

Neutron scatter from histone and DNA

We were particularly interested in the contrast variation of the low angle scattering from histone. Neutron scatter curves from films of total histone and H1-depleted histone show a clear

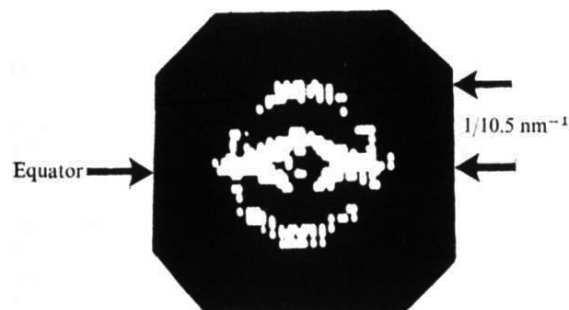


Fig. 3 Neutron scatter pattern (at 98% r.h. D₂O vapour) from an H1-depleted chromatin film which was stretched to produce orientation in the vertical direction. The pattern was reproduced on a display screen representing about 4,000 detectors of equal sensitivity over the area of the picture. The screen is 'brightened up' over a restricted range of detector counts. Wavelength, 12 Å.

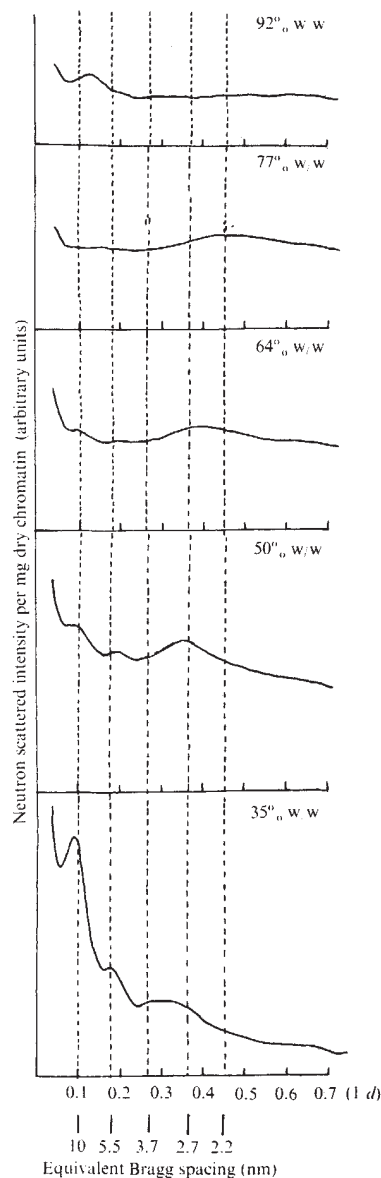


Fig. 4 Concentration dependence of the radial distribution (averaged over 360°) of neutron scattered intensity in H_2O for total chromatin. The distribution is superimposed on a background of incoherent scattering (not shown). The results for H1-depleted chromatin were very similar. The chromatin was depleted in histone H1 using an ion exchange resin³⁷ (Bio-Rad AG50W-X2) with a final buffer concentration of 0.65 M NaCl, 10 mM Tris-HCl, pH 7.

diffraction peak at 13.0 nm (equivalent Bragg spacing) in hydrated histone films. This ring is probably related to the scatter at 11.0 nm in native chromatin and reached zero intensity at about 44% D_2O content, a similar result to that obtained for the low angle scatter peak from H1-depleted chromatin mentioned above. We have also studied the contrast behaviour of films of DNA hydrated at 100% r.h. A strong neutron scatter peak corresponding to the packing of DNA behaved in a manner similar to that exhibited by the 2.7 nm peak, with change of D_2O content, shown in Fig. 2.

Discussion

The use of 'contrast matching' in low angle neutron scatter studies of chromatin shows that the low angle rings at about 11.0, 5.5, 3.7 and 2.7 nm originate not from the spacing of a single structural repeat and its higher orders (regular supercoil

model) but from different spatial arrangements of the histones and the DNA. The arrangement of histones, H2A, H2B, H3 and H4, provides a broad distribution of neutron scatter which is strong in the region of the concentration-dependent 10–11 nm peak and also has a strong component at 3.7 nm, whereas the DNA component contributes to the rings at 5.5 and 2.7 nm. Thus the region of neutron scatter to which the two main

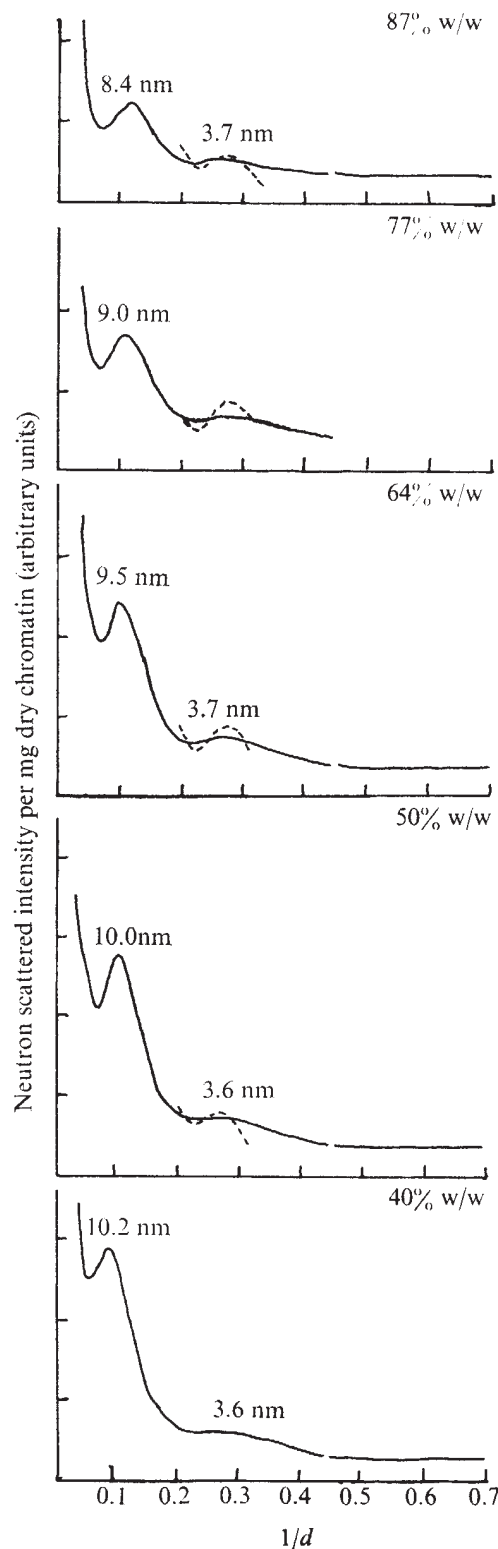


Fig. 5 Concentration dependence of the radial distribution (averaged over 360°) of neutron scattered intensity in D_2O for total chromatin. $X\%$ is the percentage concentration of dry chromatin in the films, obtained by bringing the films to a constant weight in a vacuum after the experiment. The dotted curves represent vertical magnification by a factor of 6.

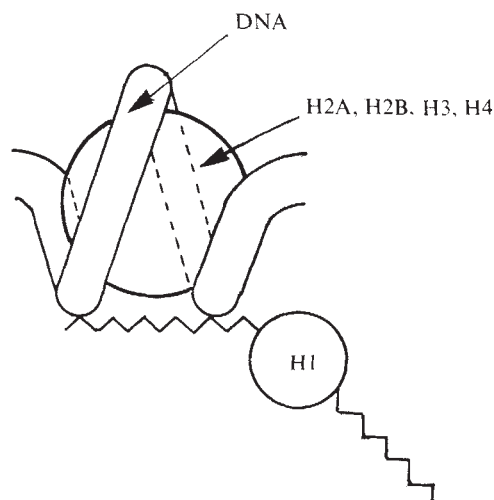


Fig 6. Schematic representation of a possible model for the chromatin subunit structure. The protein core is a complex of the apolar segment of the four histones indicated, the basic segments of the histones being complexed with DNA on the outside of the unit. Histone H1, possibly on the outside of the chain of globular subunits, may have a cross-linking role, either between subunits in the same chain or between subunits in different chains. The pitch of the DNA, which need not be uniformly coiled, is 5.5 nm with a mean diameter of about 10 nm.

components of chromatin contribute can be largely separated even though it is strictly a case of adding together all the scattering vectors of the whole structural unit to obtain intensities.

Clearly the concentration-dependent ring at about 10.5 nm arising from protein scatter can be attributed to the interparticle spacing of the proposed subunit structure for chromatin. Together with the low angle ring obtained from the neutron scatter of total and H1-depleted histone, this suggests that histones H2A, H2B, H3 and H4 comprise a multimeric protein unit, occupying a separate region of space to the DNA component. Neutron scatter data suggest that DNA is external to the histone. The equatorially oriented low angle scatter distribution (Fig. 3) extends out to equivalent spacings as high as about 4.0 nm; using an analysis analogous to Clark-Jones²⁶ this may be interpreted in terms of the equatorial transform of the individual subunits in the string. The contrast matching experiments show that this scatter and the background scatter in more dilute solution comes from the low angle component of the protein transform, which is presumably at a higher angle than the low angle component of the DNA transform. This would be expected from a globular unit containing a core of histone surrounded by DNA. It is not clear whether this protein core is spherical or extended along the fibre direction; the directional properties of this structure will involve contrast and concentration studies on oriented chromatin films to seek out zeroes in the subunit Fourier transform (*c.f.* 40).

The difference in concentration dependence of the intensities of the low angle rings in H₂O (Fig. 4) compared to D₂O (Fig. 5) could be explained by the contrast matching of the protein unit against a matrix of hydrated DNA. Histones contain highly basic segments and NMR studies show that these segments (for example, 1-31 of histone H4, 1-31 of histone H2B, 1-25 of histone H2A) are the primary sites of interaction with DNA, whereas the complementary apolar segments (in which the secondary structure can be induced by salt) are the sites of histone-histone interaction^{27,28}. Similar interaction of the apolar segments would be expected in the observed interaction between different histones²⁹. The neutron data clearly show regions of protein separate from DNA; we propose

that these regions are complexes of the apolar segments of the histones H2A, H2B, H3 and H4. When the chromatin is diluted water will preferentially hydrate the DNA; in the neutron scatter, therefore, the apolar protein cores will be contrasted against a matrix of hydrated DNA. Figure 1 shows that the protein neutron scatter density lies between that for DNA and water, only in H₂O and at low D₂O contents. As the H₂O hydration of DNA changes with concentration the scatter of the hydrated DNA, at a particular state of hydration, can therefore match that of the protein. With D₂O no such concentration-dependent contrast matching would occur because the neutron scatter densities of both DNA and D₂O are greater than that of the protein. The 3.7 nm ring, attributable largely to protein scatter, does not move during hydration. This possibly arises from the arrangement of proteins in the core of the subunit, which is unaffected by hydration. A core type subunit model has already been suggested^{18,30}.

The DNA component of chromatin at the lower concentrations contributes scatter to the rings at 5.5 and 2.7 nm; each chromatin subunit possibly contains 1.5-2 turns of a coil of DNA (pitch 5.5 nm) wound on the outside of the histone core. Other DNA folds are however possible.

A schematic representation of our model is shown in Fig. 6. A DNA subunit length of 205 base pairs could be accommodated in the model if the mean diameter of the DNA coil is about 10.6 nm. Since part of this DNA forms links between the subunit, the diameter of the DNA coil would be correspondingly reduced, depending on the length of these links. It is possible that the 170 base pair DNA unit observed¹⁸ forms part of the subunit, the additional 35 base pairs forming the link.

Very lysine rich histone H1, is not involved in the subunit structure giving the low angle rings⁷ and many studies have implicated H1 in a cross-linking role in chromatin. Further, the radius of gyration of chromatin is reduced after specific removal of H1 (ref. 32), suggesting that this histone is on the outside of the chain of globular units. The constant value for the radius of gyration of chromatin in H₂O and D₂O proposed³², is not inconsistent with this model if both H1 and non-histone proteins were located on the outside of the DNA coil. It is now clear, however, that detailed neutron scatter studies are required at about 44% D₂O.

Chromatin is thought to consist of a string of such globular units with unknown orientations. The packing of such units in the chromosome is mediated through protein (probably H1) interaction; similar interactions probably take place between isolated chromatin subunits. In this case, therefore, the diffraction pattern of the isolated units would be similar to those of native chromatin since a diffraction peak corresponding to the lowest angle ring would arise from simple aggregation of the subunits³³.

An attractive general hypothesis concerning the control of chromosome structure through the cell cycle is that chemical modification of histones are involved. With only five major histones a chemical modification of any histone will modify the interaction of that histone with DNA throughout the genome. Further, these chemical modifications affect the state of charge of basic residues, or of serines and threonines, which are located in or close to the basic segments of the histone molecules. It may be significant that in the model proposed above the basic segments of the histones are complexed with DNA on the outside of the globular unit and are thus accessible to enzyme modification or enzyme attack. Phosphorylation of histone H1 has been implicated in the initial stages of the process of chromosome condensation and control of cell division^{31,34,35}.

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letters to nature

Nature of Her X-1

IYENGAR *et al.*¹ have reported observations of hard X rays from Her X-1, which seem to set an upper limit of 10% on the pulsating component in the energy range 20 to 45 keV. I offer a simple explanation that does not require the additional source of X rays postulated by Iyengar *et al.*

In a strong magnetic field with $\omega \ll \omega_B$ (where ω is the photon frequency and $\omega_B (= eB/mc)$ is the electron gyro-frequency) the Thomson scattering cross section is reduced by $\sim (\omega/\omega_B)^2$ from its zero-field value for electromagnetic waves with E-vector pointing perpendicular to the field lines (because the electrons are constrained to oscillate along the field lines). In a magnetised plasma with $\omega \ll \omega_B$, the E-vector of the 'extraordinary' mode will essentially always be perpendicular to the magnetic field, whereas for the 'ordinary' mode this will be the case only for propagation along the field lines²: thus the reduction in the Thomson scattering cross section occurs in general only for the extraordinary mode. I now refer to the model widely used to explain the X-ray pulsing from members of binary systems (specifically Her X-1 and Cen X-3), in which a rotating neutron star with a non-aligned magnetic field accretes material from its companion, the magnetic field channelling the infalling matter toward a 'hot spot' near the surface of the neutron star³⁻⁶. As I show elsewhere⁷, the gravitational energy carried by the infalling ions is transferred to the electrons in a stationary background plasma through Coulomb collisions; these electrons then produce the bulk of the observed X-ray emission, the mechanism depending on the strength of the magnetic field at the neutron star surface, B_0 . Should this field be so strong that $\hbar\omega_{B_0} \sim kT_e$, then the quantisation of the electron orbital energy must be taken into account. In particular, if $kT_e < \hbar\omega_{B_0}$ the only state energetically allowed to most electrons is the ground state, so that these electrons are constrained to move parallel or antiparallel to the field lines, and cannot emit cyclotron radiation.

It may now be argued that photons with frequencies $\omega \ll \omega_{B_0}$, produced at the base of the accretion funnel, will be markedly beamed in a direction perpendicular to the field lines. They are emitted by nonrelativistic electrons undergoing one-dimensional bremsstrahlung, so that the original radiation pattern is dipolar with maximum intensity perpendicular to the field lines (note that the photon frequencies far exceed the plasma frequency, and that the emitted photons are predominantly linearly polarised and in the ordinary mode); and the scattered radiation

pattern will be similar. Furthermore, even though the Thomson scattering cross section is reduced for both modes propagating along the field lines, a photon is not likely to escape upward through the accretion funnel, not only because it encounters far more material in that direction, but also the mean free path against Thomson scattering rapidly decreases in that direction because of the fall-off of the magnetic field, so that the photon tends to random walk downwards. Indeed, if one assumes that within the accretion funnel the plasma flows along the field lines at the free-fall velocity, then the steady state continuity equation yields

$$n(r) \sim n(R)(r/R)^{1/2} [B(r)/B_0] \quad (1)$$

where r denotes radial distance from the centre of the star and R the radius of the star, so that for reasonable magnetic fields $B(r)$ the fall-off of density in the accretion funnel, $n(r)$, is sluggish compared to that in, for example, the emitting region itself⁷. On the other hand, above the emitting region, the scattering cross section increases as $\sim (\omega/\omega_B)^2$, so that the photon mean free path decreases as

$$l_{\parallel}(r) \sim l_{\parallel}(R)(R/r)^{1/2} [B(r)/B_0] \quad (2)$$

until $B(r) \rightarrow \omega m_e c/e$ and the Thomson scattering cross section relaxes to its zero-field value, at which point the photon mean free path begins to increase inversely as $n(r)$. As an example, for a dipole field $B(r) \propto r^{-3}$, one has $n(r) \propto r^{-5/2}$ and $l_{\parallel}(r) \propto r^{-7/2}$. The beaming will be further enhanced through the conversion of ordinary into extraordinary mode photons in some scatterings, the latter then escaping easily if they are directed approximately perpendicular to the axis of the accretion funnel. (A more detailed investigation of photon diffusion through plasma in a superstrong magnetic field is being carried out by M. Rosenberg and Y.-M.W.) It is then clear that the radiation must emerge near the base of the accretion funnel in a markedly fan-shaped beam (exhibiting, incidentally, substantial linear polarisation).

On the other hand, at higher frequencies, that is for $\omega \rightarrow \omega_{B_0}$, the electrons are no longer constrained to move one-dimensionally while the Thomson scattering cross section rises to its zero-field value for both modes of propagation, and the beaming should be at least partially smeared out. (The magnitude of this effect will depend on, for example, the density gradient transverse as compared to that parallel to the axis of the accretion funnel.) I assume that the pulsating component of the Her X-1 emission vanishes between 20

and 45 keV, and obtain a rough estimate of the magnetic field at the star surface by setting $20 \text{ keV} \lesssim \hbar\omega_{B_0} \lesssim 45 \text{ keV}$, from which $2 \times 10^{12} \text{ gauss} \lesssim B_0 \lesssim 4 \times 10^{12} \text{ gauss}$. Interestingly, Iyengar *et al.* claim that the data for the entire energy range 2–80 keV can be fitted by a bremsstrahlung spectrum with a temperature of about 23 keV. On the basis of the above considerations, one would expect a bremsstrahlung spectrum below $\sim \hbar\omega_{B_0}$; although the shape of the spectrum above $\sim \hbar\omega_{B_0}$ is difficult to determine, one might expect a steep fall-off because of cyclotron emission.

I emphasise that the absence or near absence of hard X-ray pulses from Her X-1 claimed by Iyengar *et al.* is as yet unconfirmed by any other group. Indeed, Holt *et al.*⁸ find that the Her X-1 pulsed emission above $\sim 12 \text{ keV}$ is characterised by narrower peaks than that at lower energies (with no apparent difference in the ratio of pulsed to non-pulsed emission); in addition, they find a sharp cutoff in the (total) spectrum at $\sim 24 \text{ keV}$ which seems irreconcilable with the high energy data of Iyengar *et al.* If the surface field on Her X-1 is relatively weak, so that $\omega > \omega_{B_0}$ over the observed range of energies, then the dominant emission mechanism would be magneto-bremsstrahlung at a rate determined by the collision frequency (since the cyclotron decay time is far shorter than the Coulomb deflection time for an electron) giving rise to a soft energy spectrum⁷; and in this case the pulses at low energy might well be somewhat broader than those at higher energy because of the emission of soft X rays above the star surface (for example, cyclotron emission by electrons spiralling down the accretion funnel).

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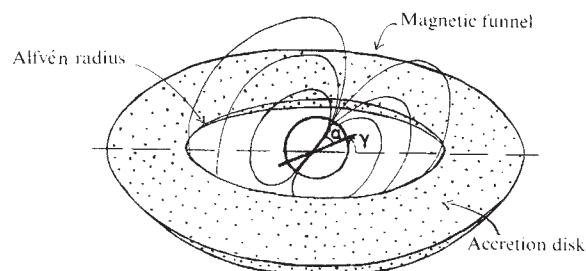


Fig. 1 Diagram of Her X-1 with accretion disk. Magnetic funnel is shown here out of accretion plane. Neutron star is not to scale.

has been developed in support of this model⁴. But it is not clear that some proposed alternative models^{5–7} can account for the requisite narrow beam width. Beaming in these models is difficult if the magnetic moment of the neutron star is greater than $2 \times 10^{29} \text{ gauss cm}^3$ (ref. 8). The first assumption is thus open to question.

The second assumption is called into question by the possibility that the amount of matter falling into the accretion column will vary within each period. The accreting matter spirals into the neutron star in a thin plane until it is caught by the magnetic 'funnel' at the Alfvén radius⁸ ($R_{\text{Alf}} \approx 10^8 \text{ cm}$), from which it is accelerated directly on to the surface. For most choices of α and the angle between the rotation axis and the accretion plane (γ) the magnetic funnel will not lie continuously in the accretion plane but will sweep in and out of it during each rotation (Figs 1 and 2). Since the plasma density in the accretion plane is much greater than the density of matter distributed generally around the magnetosphere, one would expect well defined spurts of dense plasma to fall down the accretion column as the funnel passes through the plane. If these density variations arrive at the base of the accretion column intact, the X-ray emission will be pulsed. For many values of α and γ the funnel will pass through the accretion plane twice in each period, thereby providing for the double-peaked pulse structure (Fig. 2a).

So I propose that the 1.24 s pulses originate from luminosity variations at the source arising from time-varying accretion rather than from the passage of a beam of constant intensity past the Earth.

Unlike the conventional model, my model does not depend on any particular emission mechanism or beam width, puts no restriction on β , and takes cognisance of the anisotropic distribution of accreting matter around the magnetosphere.

This model is only feasible in certain conditions. First, as with the standard model, α cannot be 0° . This condition is met by most known neutron stars. Second, in order for the magnetic funnel to move in and out of the accretion plane, γ must not be 90° . Though it is frequently claimed that $\gamma = 90^\circ$, my condition will hold if the rotation axes of Her X-1 and HZ Herculis, the companion star, are not aligned. They might have been misaligned before the collapse of Her X-1 or γ might have been changed during the collapse. It has further been suggested that if HZ Her were once illuminated unevenly by X rays from Her X-1, the accretion disk would permanently lie out of the orbital plane, resulting in $\gamma \neq 90^\circ$. Two forces might be thought to force the rotation axis perpendicular to the accretion disk: relativistic frame dragging and torque exerted by the accreting matter. The frame-dragging effect will significantly alter the orientation of the disk only if $R_{\text{Alf}} \lesssim 2 \times 10^7 \text{ cm}$ (ref. 10). The torque will be directed against the magnetic axis rather than the rotation axis. Thus, unless the magnetic and rotation axes align themselves or the neutron star wobbles, the magnetic funnel will still sweep in and out of the accretion plane during each rotation.

Next, it is assumed that the movement of the magnetic funnel through the accretion plane does not seriously distort

Model for 1.24 s X-ray pulses in Her X-1

THE 1.24 s X-ray pulses observed in the 2–20 keV region from Her X-1 are thought to arise from matter being accreted on to a rotating, magnetic neutron star. The pulse shape has been studied by several groups^{1–3}. More than 65% of the emission is concentrated in 0.4 of the duty cycle. The emission in this interval is usually double peaked, though it is sometimes single peaked¹ or asymmetrical^{2,3}. The pulse profile varies on time-scales of hours to days¹, and from pulse to pulse (my unpublished work with T. Chetani and R. Giacconi).

The pulsed emission is usually thought to be produced by a process analogous to that of radio pulsars: X rays are emitted in a directed fan beam from the magnetic poles of the neutron star, which scans a circle in the sky as the star rotates. The Earth is in the line-of-sight of the beam only during a portion of each 1.24 s period. The double-peaked feature can be accounted for qualitatively by assigning particular values to the angle between the rotation axis and the magnetic axis (angle α), the angle between the rotation axis and the line-of-sight (β), and the width of the emitted fan beam². This model relies on two important assumptions: highly anisotropic X-ray emission from the base of the accretion column (beam width $\approx 25^\circ$), and constant luminosity at the source. A theory of highly beamed emission caused by synchrotron processes

the structure of the accretion disk. This requirement can be verified only when more is known about the interactions at the Alfvén radius.

Finally, this model imposes some conditions on the properties of the plasma in the accretion column. Limits on turbulence and convection during the descent are required so that variations in the density of the infalling plasma are not smoothed out beyond the timescale of features observed in the Her X-1 pulse profile (rise time ≈ 50 ms). Since the total free-fall time from R_{Alf} to the surface of the neutron star is ≈ 100 ms, this condition would be violated only by very largescale effects in the column or by significant delays at shock fronts or deceleration zones. The model further demands that each spurt of plasma cool within ≈ 50 ms. This has been substantiated by preliminary calculations⁸.

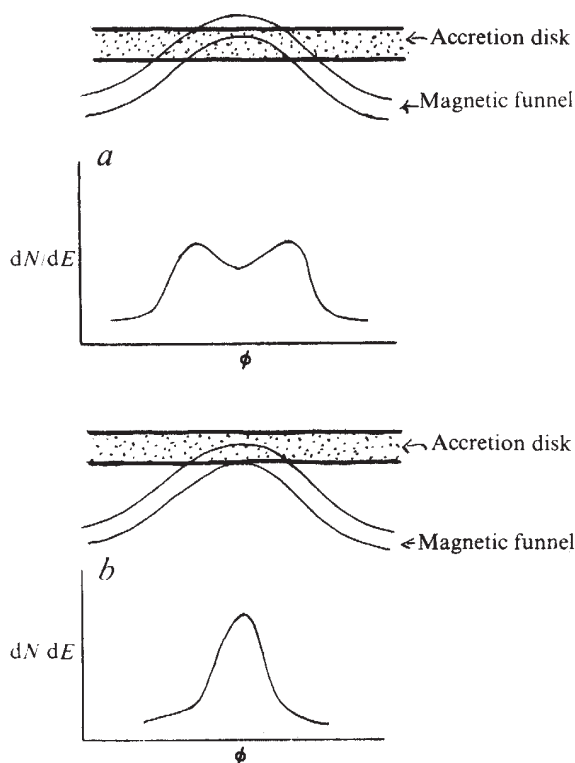


Fig. 2 Relative position of magnetic funnel and accretion disk as a function of phase, and the resulting pulse shape: a, double-peaked pulse; b, single-peaked pulse after precession of the neutron star or accretion disk.

None of the conditions listed here seem insurmountable, though several may warrant further investigation.

Since the rotation axis and the accretion plane are not perpendicular in this model, either the neutron star or the accretion disk will be precessing. Precession has been proposed by several authors as the source of the 35-d periodicity of Her X-1^{8,9,11}. Evidence for variation in the shape of the 1.24 s pulse across the 35-d cycle would provide strong evidence for the present model. Qualitatively, it predicts the secondary minimum to be more pronounced in the middle of the 9-d 'on' state, and more single-peaked pulses to be observed at the beginning and end of each 'on' state (Fig. 2a and b).

Short term variations in pulse shape and intensity (≈ 1 –10 s) observed in Uhuru sightings (unpublished) of Her X-1 may be explained in this model in terms of fluctuations in accretion disk density. The fluctuation frequency is expected to be roughly equal to the orbital period at R_{Alf} , $t \approx 2\pi (GM/R_{\text{Alf}}^3)^{1/2} \approx 1$ s (ref. 10), as observed. This model, like the standard

model, cannot easily account for reported asymmetries in the pulse profile.

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Cosmological effects of primordial black holes

ALTHOUGH only black holes with masses $\gtrsim 1.5M_{\odot}$ are expected to result from stellar evolution¹ black holes with much smaller masses may be present throughout the Universe². These small black holes are the result of density fluctuations in the very early Universe. Density fluctuations on very large mass scales were certainly present in the early universe as is evident from the irregular distribution of galaxies in the sky³. Evidence of density fluctuations on scales smaller than the size of galaxies is generally thought to have been destroyed during the era of radiation recombination⁴. But fluctuations in the metric of order unity may be fossilised in the form of black holes. Observation of black holes, particularly those with masses $M < M_{\odot}$, could thus provide information concerning conditions in the very early Universe.

One indication that many black holes exist at present in the Universe is the evidence that the average density of matter in the Universe greatly exceeds the observed density of matter. Application of the virial theorem to galactic clusters⁵ implies that the density of matter is at least five times the observed density. Measurements of the deceleration⁶ and positions³ of galaxies suggest that the density of matter may be larger than the observed density by a factor ~ 100 —perhaps enough to make the Universe closed, although these measurements are rather uncertain. On the other hand, there are reasons⁷ for believing that the observed deuterium in the Universe was formed in the early radiation era. This would place an upper limit on the free nucleon density during the first 15 min of the Universe. This upper limit on the nucleon density implies that the present-day matter density $\lesssim 6 \times 10^{-31} \text{ cm}^{-3}$; that is, ~ 10 times the observable matter density. This upper limit on the total matter density would be consistent with applications of the virial theorem to galactic clusters but would not be consistent with the above-mentioned measurements suggesting a higher density. If evidence for a cosmologically flat or closed Universe holds up then it follows that during the first 15 min most of the matter in the universe must have existed in some other form than free nucleons—in other words, black holes.

If many small black holes ($M < M_{\odot}$) exist at the present time then their presence may be revealed because they radiate electromagnetic radiation. Indeed, during collapse the metric will be changing rapidly on a time scale $\tau \approx 10^{-5} (M/M_{\odot})$ s, so that production of massless particles with energy of order $\hbar/\tau$ is expected⁸. Thus masses smaller than about 10^{20} g will radiate X rays and gamma rays when they undergo gravitational collapse. Hawking⁹ has suggested that the emission of massless

particles can be interpreted by saying that black holes have a temperature $\sim 10^{-6} (M_{\odot}/M)$ K. This interpretation implies that black holes with masses as large as 10^{15} g would have radiated away all their mass by now. Davies and Taylor¹⁰ have, however, suggested that the emission of radiation from a black hole only takes place for a brief instant during the collapse and that only black holes with mass $\lesssim 10^{-4}$ g will radiate away a significant fraction of their mass. If they are correct then practically all radiation due to black holes would have been emitted in the very early Universe and at the present time would only show up as a possible contribution to the 3 K microwave background.

On the other hand, if Hawking's interpretation is correct then small black holes would have produced X- and gamma-radiation up to and including the present time. If the black hole mass spectrum is not varying too rapidly the result would be background radiation the spectrum of which started out as a continuation of the 3 K blackbody spectrum and rose slowly to a peak at an energy determined by the smallest black hole mass now existing. Observational evidence for Hawking's interpretation might be provided by a distortion in the 3 K blackbody spectrum for wavelengths < 1 cm or by a peak in the isotropic X-ray background above 10 MeV (corresponding to Hawking's estimate of 10^{15} g as the smallest black hole mass now existing). A flattening of the isotropic X-ray background spectrum at about 30 MeV has, in fact, been reported¹¹. If we identify the measured X-ray flux associated with this feature ($\sim 10^{-6}$ photons $\text{cm}^{-2} \text{s}^{-1} \text{keV}^{-1}$) with X-ray emission from black holes with masses in the neighbourhood of 10^{15} g then one arrives at a space density of these black holes $N_{\text{max}} \approx 10^{-52} \text{cm}^{-3}$. Because the present-day spatial density of galaxies is about 10^{-75}cm^{-3} we conclude that there may be as many as 10^{23} small black holes per galaxy. If we assume that the volume of our Galaxy is 10^{12}pc^3 then a region the size of our Solar System ($\sim 10^{44} \text{cm}^3$) might contain several small black holes. Of course, there are other possible interpretations^{12,13} of the 30 MeV feature in the X-ray background and therefore the actual spatial density of small black holes may be much less than N_{max} .

In order to calculate the contribution of small black holes to the total mass of the Universe one must make some assumption about the primordial black hole mass spectrum. The simplest assumption that can be made is that the number of black holes in each logarithmic interval of mass is the same:

$$dN = N_0 dM/M \quad (1)$$

This assumption is consistent with the idea¹⁴ that perturbations of the metric in the early Universe should be independent of scale length.

With the mass spectrum (1) the total mass of primordial black holes is $N_0 M_{\text{max}}$ where M_{max} is the maximum mass of a primordial black hole. Taking $N_0 = N_{\text{max}}$ and a present-day mass density $\lesssim 10^{-30} \text{g cm}^{-3}$ gives $M_{\text{max}} \lesssim 10^{22} \text{g}$. This maximum mass for primordial black holes may be related to the Hubble mass at the time when black hole formation in the early Universe stopped. It is interesting that the Hubble mass was equal to 10^{22}g at some time during the hadron era ($t < 10^{-4} \text{s}$) when large density fluctuations are 'predicted' to occur in some theories of dense hadronic matter¹⁵. Conversely, identification of any of the cosmological effects we have discussed as being the result of small black holes should provide some information on the nature of dense hot hadronic matter. A search for evidence of small black holes in the Solar System might be very worthwhile. Indeed, the existence of small black holes in the Solar System might have considerable economic significance because small black holes would be very useful as power sources¹⁶.

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ATS-6 radio beacon experiment

SINCE ATS-6 was launched into a geostationary orbit at 94°W in late May 1974, measurements of the total electron content (TEC) using the Faraday polarisation-rotation¹ and group dispersive-delay techniques have been made at Fort Monmouth (40.18°N; 74.06°W). Comparison of TEC rate of change obtained by the two techniques yields the temporal variation of the integrated number of free electrons above the ionosphere. This variation indicates a flow of electrons from regions above the ionosphere into the ionosphere at night, while during the day the direction of flow is reversed. The rate of the electron flux is estimated using the continuity equation.

The total Faraday¹ rotation from the signal source to the observer is related to the total electron content by the expression:

$$\begin{aligned} a &= (k/f^2) \int B \cos \theta N ds \\ &= (k/f^2) \int (B \cos \theta \sec \chi) N dh \\ &= (k/f^2) \bar{M} N_1 \end{aligned} \quad (1)$$

where $k = 2.36 \times 10^{-5}$, M is the magnetic field factor (at 420 km), N_1 is the total ionospheric electron content, and $f = 140$ MHz. As B decreases inversely with the cube of the geocentric distance and the electron density decreases exponentially with altitude above F_2 (max) (~ 300 km), the rotation is heavily weighted near the Earth and is considered to provide electron content values below $\sim 1,200$ km.

Using the dispersive-group-delay technique², the phase of the modulation envelope between a carrier and its sideband is compared at two frequencies (nominally $f_{1,2} = 140, 360$ MHz with a sideband displacement of $\Delta f = 1$ MHz). Since the phase is insensitive to the Earth's magnetic field, this technique yields the number of electrons along the entire path from satellite to observer (N_T).

The differential modulation phase $\Delta\phi$, in degrees, is:

$$\Delta\phi/360 = 40.3 \Delta f/c \sec \chi (f_1^{-2} - f_2^{-2}) N_T \quad (2)$$

where c is the speed of light *in vacuo*, and χ is the zenith angle.

The relative variation of the total electron content measured by the Faraday and group-delay techniques is shown in Fig. 1a and b at 15-min intervals for the time period 1600 EDT on July 3 to 0800 EDT on July 8. The temporal variations of N_1 and N_T were nearly parallel with most density variations observed on both curves. In general, $\Delta N_1/\Delta t$ and $\Delta N_T/\Delta t$ varied between $\pm 1 \times 10^{16}$ electrons m^{-2} per 15-min interval.

Large increases of total electron content, in response to two large solar flares, are prominent during the time period covered by Fig. 1. Between 0945 EDT and 1000 EDT on July 4, N_1 increased by $\sim 1.5 \times 10^{16}$ electrons m^{-2} , while N_T increased by $\sim 2 \times 10^{16}$ electrons m^{-2} . Since the content values in Fig. 1a and b are given every 15 min, the full increase of N_1 and N_T is not indicated there. Starting at ~ 0953 EDT, N_1 increased by $\sim 3.3 \times 10^{16}$ electrons m^{-2} in 3 min and then decayed to its figure value at 1000 EDT. At the same time, N_T increased by approximately

the same amount. On July 5 between 1730 and 1745 EDT, N_I increased by $\sim 1.9 \times 10^{16}$, while N_T increased by $\sim 2.3 \times 10^{16}$. The rapid increases started at ~ 1740 EDT with N_I increasing by $\sim 2.1 \times 10^{16}$ in ~ 6 min; N_T increased similarly.

The dispersive-group-delay technique measures the total electron content from observer to the geostationary satellite, whereas the Faraday technique yields the content only in the vicinity of the Earth (up to $\sim 1,200$ km). The difference between the two yields is the content above $\sim 1,200$ km, which is referred to as the plasmaspheric content, N_P .

It follows that the rate of change of the plasmaspheric content is:

$$\Delta N_P/\Delta t = \Delta N_T/\Delta t - \Delta N_I/\Delta t \quad (3)$$

The rate of change $\Delta N_P/\Delta t$ at 15-min intervals is plotted in Fig. 2a and b for the same time period as Fig. 1. The absolute values of $|\Delta N_T/\Delta t| - |\Delta N_I/\Delta t|$ for either positive (N_I and N_T both increase) or negative (N_I and N_T both decrease) are indicated. A negative value for either of the two cases indicates that the N_I variations was larger than the N_T variation. This must indicate a flow of electrons between the plasmasphere and the ionosphere, which would change N_T by a lesser amount than the corresponding change in N_I .

Figure 2 shows that, for the most part, the changes of the plasmaspheric content at 15-min intervals were generally restricted to $\pm 0.5 \times 10^{16}$ electrons m^{-2} .

As expected the rate of change of N_T was more often than not larger than the rate of change of N_I (all positive values in Fig. 2). We expect this because N_I is part of N_T , and any changes in N_I will be reflected in N_T . An interesting behaviour of $\Delta N_P/\Delta t$ occurred during the decay phase of the content on July 5–6, July 6–7 between ~ 2000 and 0430 EDT, when it was generally positive indicating that the total content was decreasing faster than the ionospheric content. Since loss of plasmaspheric content through recombination cannot account for the difference between $\Delta N_T/\Delta t$ and $\Delta N_I/\Delta t$, the only plausible

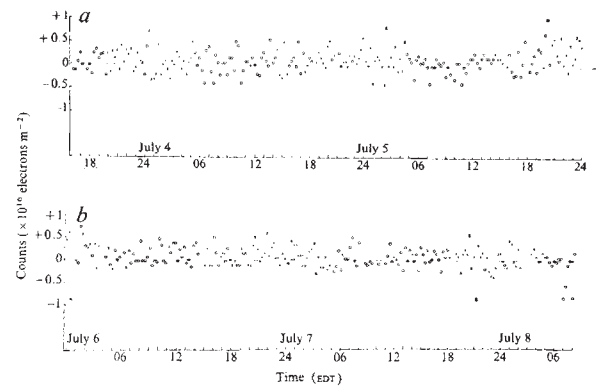


Fig. 2. Variation of plasmaspheric electron content ($N_T - N_I$). $\circ$, variation for $N_T, N_I > 0$; $\times$ variation for $N_T, N_I < 0$. A negative value indicates $\dot{N}_I > \dot{N}_T$.

cause for the difference is electron flux from the plasmasphere to the ionosphere.

I suggest the following mechanism. The ionospheric electron content decreased sharply which resulted in equal decreases of N_T and N_I . With the corresponding fall in electron temperature, ionospheric layer collapse induced electron fluxes from the plasmasphere to the ionosphere. Although these fluxes did not alter the value of N_T , since they were still measured by the group delay technique, they did slow the rate of decrease of N_I because they reached lower altitudes where they were then measured by the Faraday rotation technique. Increases in total ionospheric electrons^{3–5} can be seen with the Faraday technique only when the flux rate exceeds the recombination rate, indicating an actual increase in ionospheric total density. But with the combination of Faraday and group-delay techniques, made possible by the radio beacon experiments of ATS-6, such fluxes may be determined even when their rate is smaller than the decay rate due to recombinations.

Assuming electron transport in the vertical direction only, the magnitude of the fluxes can be estimated.

The rate of change of N_I can be expressed by the continuity equation

$$\Delta N_I/\Delta t = q_I - L_I - F \quad (4)$$

where q is the production rate, L is the loss rate ($L = \int_{\text{Ionos.}} \beta(h) N(h) dh$ where $\beta(h)$ is the loss rate coefficient) and F is the flux term. Similarly for N_T

$$\Delta N_T/\Delta t = q_T - L_T \quad (5)$$

The flux term has been omitted here since any fluxes between ionosphere and plasmasphere will still be measured by the group-delay method. Since production and loss are effective only at ionospheric heights, it follows that $q_T \approx q_I$, $L_T \approx L_I$, and therefore

$$\Delta N_T/\Delta t - \Delta N_I/\Delta t \approx F \quad (6)$$

using the results of Fig. 2, the estimated fluxes are $\lesssim \pm 0.5 \times 10^{13}$ electrons $m^{-2} s^{-1}$.

Particle fluxes also play a role during the build-up phase. For example, between 0400 and 1400 EDT on July 6, $\Delta N_P/\Delta t$ was generally positive indicating that N_T increased faster than N_I . Electron production cannot contribute to an increase in N_P , but an increase in the particle temperature expands the ionospheric layers and thus causes electron fluxes from the ionosphere to the plasmasphere, increasing the content of the latter. N_T

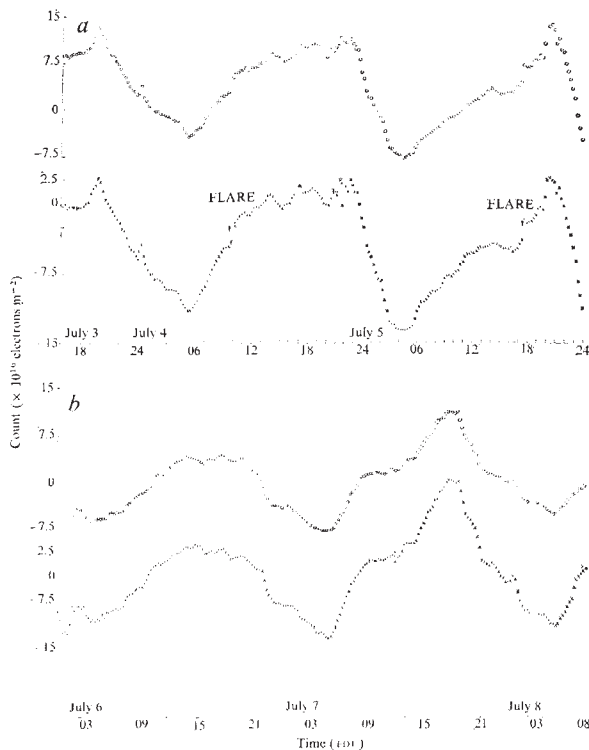


Fig. 1. Relative variation of total electron content, N_T ($\circ$), and ionospheric electron content, N_I ($\times$) at 15-min intervals 1600 EDT, July 3, 1974, to 0800 EDT, July 8, 1974, at Fort Monmouth, New Jersey.

reflects the increase in production in the ionosphere and electrons in the plasmasphere due to these fluxes. The rate of increase of N_1 is slower since the fluxes slow down the rate of increase by electron production.

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Effect of vorticity pollution by motor vehicles on tornadoes

THE recorded annual incidence of tornadoes in the United States has increased steadily and dramatically in the past four decades, by at least a factor of six¹. We have examined the thesis that the development of widespread motor vehicle traffic in the United States over the last 40 yr has perturbed atmospheric vorticity in such a way as to exacerbate the tornado incidence, principally by the introduction of cyclonic vorticity. Surprisingly, both the analysis and the evidence support the thesis.

Tornadoes depend on non-random vorticity in strong convection and motor vehicle traffic produces a unique anthropogenic input of non-random vorticity into the troposphere.

Motor traffic on the North American continent passes opposing traffic on the right. The thrust of each motor vehicle against the solid Earth is thus ordinarily to the right of that of the opposing traffic. To the extent that these thrusts result from momentum transfer to the atmosphere, a plethora of additive anticyclonic force couples act on the Earth's crust, and cyclonic torque is applied to the atmosphere. These effects persist and accumulate, limited only by the exchange of the atmospheric vorticity with the solid Earth, which according to our calculations occurs slowly. It is this anthropogenic addition to atmospheric cyclonic vorticity that might constitute a link between motor vehicle traffic and tornadoes. We are here neither contemplating the temporary turbulence in the wake of passing vehicles, nor the angular momentum stored in the vehicles underway, but rather the interaction of the opposed atmospheric momentum streams engendered by two opposed streams of traffic (Fig. 1).

In the USA there are $\sim 2 \times 10^6$ automobiles and 6×10^5 trucks underway at any average moment². Cyclonic vorticity introduced into the troposphere by US traffic in one week is much greater than the Earth's vorticity, and annually is of the order of 10^5 – 10^6 times the vorticity involved instantaneously in a single tornado. Trucks and automobiles contribute equally. In our calculation we used an average spacing of 20 m between opposed traffic streams. The magnitude of the torque is of course dependent on this number, and recent increased spacing between lanes has increased the total torque, but a reasonable range of spacings would affect the torque by less than one order of magnitude.

In our model, the continent was initially enveloped in a tropospheric air mass, drifting west to east, that contained an overall cyclonic vorticity with substantial regions of no or anticyclonic vorticity. With the increase of motor vehicle traffic, the air masses were treated with increasing cyclonic vorticity, which cancelled much of the anticyclonic vorticity and produced: (a), a more homogeneously cyclonic troposphere; (b), more numerous (and more dominantly

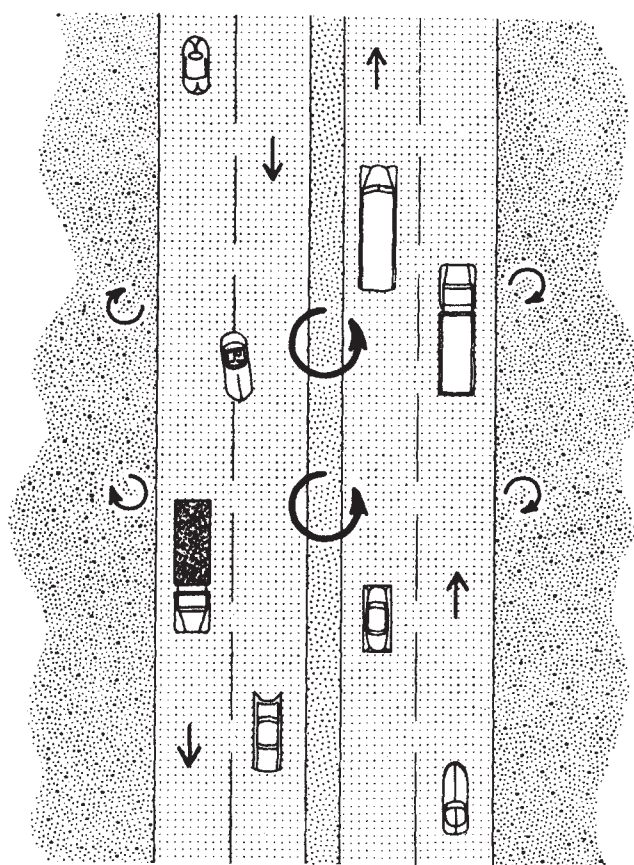


Fig. 1 "For they have sown the wind, and they shall reap the whirlwind . . ." Hosea 8:7. The cyclonic vorticity introduced into the atmosphere is generated by the torque between the two opposing streams of traffic. The smaller side vorticities created at the periphery of the highway are boundary effects eventually acting at large distances (perhaps finally at the antipodes, where they are also cyclonic).

cyclonic) tornadoes; (c), a migration of the "centre" of tornado intensity to the east; and (d) secular and periodic variations of tornado incidence reflecting major changes in motor vehicle traffic (for example, weekly). The records indicate that these phenomena have occurred.

We have obtained the detailed record of all US tornadoes (15,234) reported between 1950 and 1873 in the 48 contiguous states³. The annual incidence shows an increase over the period of a factor of four. Changes in observation and reporting are undoubtedly serious and we have applied tests that are relatively independent of secular changes in reporting.

The annual centre of tornado intensity weighted by the area of each tornado path has varied considerably and has increasingly occupied the eastern portion of the continent in the second half of the record, as our thesis predicts.

Pollution by cyclonic vorticity should have lowered the relative incidence of anticyclonic tornadoes, and this has occurred. In the first half of the record (1950–61) 4 out of 11 events for which sense was recorded were anticyclonic, whereas in the second half, this was true for only 10 out of 123. A random selection of 11 events from a distribution similar to that of the second time period would have approximately a 7.5×10^{-3} chance of recording four or more anticyclonic tornadoes.

The data display a strong weekly periodicity.

Over the entire record the reported number of tornadoes on Saturdays is less than the daily average by more than 7.1 standard deviations (1,868 on Saturdays as against a daily average of 2,176). The statistical probability of this record occurring by chance from a random sample is $P < 10^{-9}$. This hebdomadal event dominates in 18 of the 24 yr and in most

states. (Thirty-seven of the forty-eight states show a Saturday incidence below the average, $P \sim 10^{-4}$). Nineteen states show Saturdays incidence to be a minimum ($P = 1.5 \times 10^{-3}$).

We attribute this weekly periodicity to the periodicity of vorticity introduction by motor vehicle traffic. We attribute the general Saturday minimum to the fact that most truck and commuter traffic ceases on a Friday, and to the unidirectional character of the weekend flow, both in and out of urban areas, which probably introduces mainly a momentum stream into the atmosphere on Saturdays, generating net cyclonic vorticity later and downwind by interaction with the momentum stream of the traffic returning on Sunday. Other effects such as a weekend diminution of heat or particle production by industry and motor vehicles or of the efficiency of reporting are difficult to reconcile with average tornado incidence on Sundays in most states.

We would expect the strongest hebdomadal fluctuations to occur in states dominated by fresh, relatively unsullied oceanic air masses and thus influenced more sharply by the immediate input of vorticity. Indeed, coastal states display an enhanced weekly cycle with 12 of the 21 coastal states showing the Saturday incidence of tornadoes to be a minimum, and 19 of the 21 showing Saturdays to be below average in tornado occurrence (as opposed to seven showing Saturday minima and 18 showing Saturdays below average in the 27 interior states). These statistics for the special class of coastal states have 0.04 probability of being a chance selection from the statistics of all states. This test of our thesis should be independent of general reporting peculiarities, and so is crucial evidence in its support.

Additionally, as the thesis predicts, far western coastal states display a special selection of the statistics of all coastal states with minima for both Saturdays and Sundays. No far western state is subject to many tornadoes (also perhaps in support of our thesis) and, in the extreme case of the state of Washington, of the 25 tornadoes reported in the past 24 yr, none occurred on Saturday or Sunday ($P \sim 2.2 \times 10^{-4}$).

Vorticity is a unique requirement for the formation of tornadoes and hurricanes, and motor vehicles are the only man-made source of non-random vorticity we know. From our initial findings we provisionally assign an increase in reported US tornadoes to vorticity pollution by motor vehicles. If this is the case, mankind, excluding mainly the British, may need to take responsibility for yet another deleterious environmental effect. But vorticity pollution could be turned to benefit. If the obvious turnabout were made, there might well be an immediate and secular decrease in tornadoes, perhaps to levels below the natural intensity, duration and frequency.

Regardless of the veracity of our thesis, some anthropogenic effect clearly has resulted in about 300 fewer tornadoes reported in the US on Saturdays than on other days of the week, in the past 24 yr. If this results from the reduction of some human activity on Saturday (other than tornado reporting), then at least 14% of US tornadoes are under man's control. The total of man's effects could, of course, be much larger since an accumulating diffuse reservoir of vorticity might account for more of the long term (600%) increase in tornado incidence (or even changes in hurricane incidence, path and intensity) in the Northern Hemisphere.

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Diachronism of depositional and diastrophic events

ONE of the fundamental principles of geology—'Stille's legacy'¹—is the concept that there is a wide contemporaneity of geological events, both tectonic and sedimentary. The concept received notable attention 25 yr ago and raised a scientific controversy². It is now of interest again because of the present tendency to assume as a causal mechanism of mountain building the relative motions between plates. On the basis of geological evidence it has been shown³ that the diastrophic events of the western Mediterranean do not occur at the same time in the different mountain chains, because collisions between plates are diachronous. Detailed stratigraphical analysis of the units within the Maghrebain Chain⁴ (from Gibraltar to Calabrian Arc) indicates that, in addition, the initiation of geological events varies from place to place in the same orogen⁵.

The Maghrebain Fold Belt was produced largely by the tectonic deformation of three groups of Oligo-Miocene flysch (the 'tectonic triad of flysch types'⁵) (see Fig. 1).

One group is represented by a Nile Cone-type^{5,6} 'passive margin' sequence which terminates in the upper part with an alternation of quartz-rich sandstones and iron-rich silty clays. In Sicily these African-derived⁷ miogeoclinal quartzose deposits derived from Africa, comprise typical submarine fan facies (the 'external' Nebrodian Flysch and the 'internal' Numidian Flysch) and abyssal plain facies (the Malia Flysch). The basal strata of these units are transgressive northwards over multicoloured marls (scaglia-type formation⁵) and clays (*Argille varicolori*⁵) interpreted as deep-water basinal facies. The northward fan progradation occurred with a biostratigraphically estimated rate of about 10–20 km Myr⁻¹ (Fig. 2).

The second contemporaneous flysch group (Hellenic Trench-type suite^{5,6}) comprises marly-arenaceous, compositionally-immature turbidites (Tusa Flysch). The marls are homogeneous ('homogenites') and probably originate from ponding by turbidity currents^{5,6}. The sandstones are lithic (clastics from granitic-metamorphic and sedimentary sources) with sometimes significant andesitic components⁸. The Tusa Flysch sequences constitute successive thrust sheets, each sheet less than 800 m thick and progressively younger southwards. This tectonic juxtaposition is inferred to be the result of subduction under the Calabrian Massif of original trench-filling sediments^{3,5}. Migration of the trench axis occurred at only about a fifth of the rate of progradation of the Nile Cone-type flysch (Fig. 2).

The third member of the 'triad' (the Crete Basin-type flysch⁵) is a thick, tectonically undeformed, arenaceous sequence which is transgressive on the Calabrian Massif in the internal (northern) zones and overlies the tectonically deformed Tusa trench-fill deposits in the external (southern) zones. This neo-autochthonous sedimentation (Reitano-Capo d'Orlando Flysch) is believed to be an arc-trench gap accumulation⁹ that advanced progressively southwards over the deformed trench deposits accreted to the front of the Calabria microplate^{3,5}. Sicilian

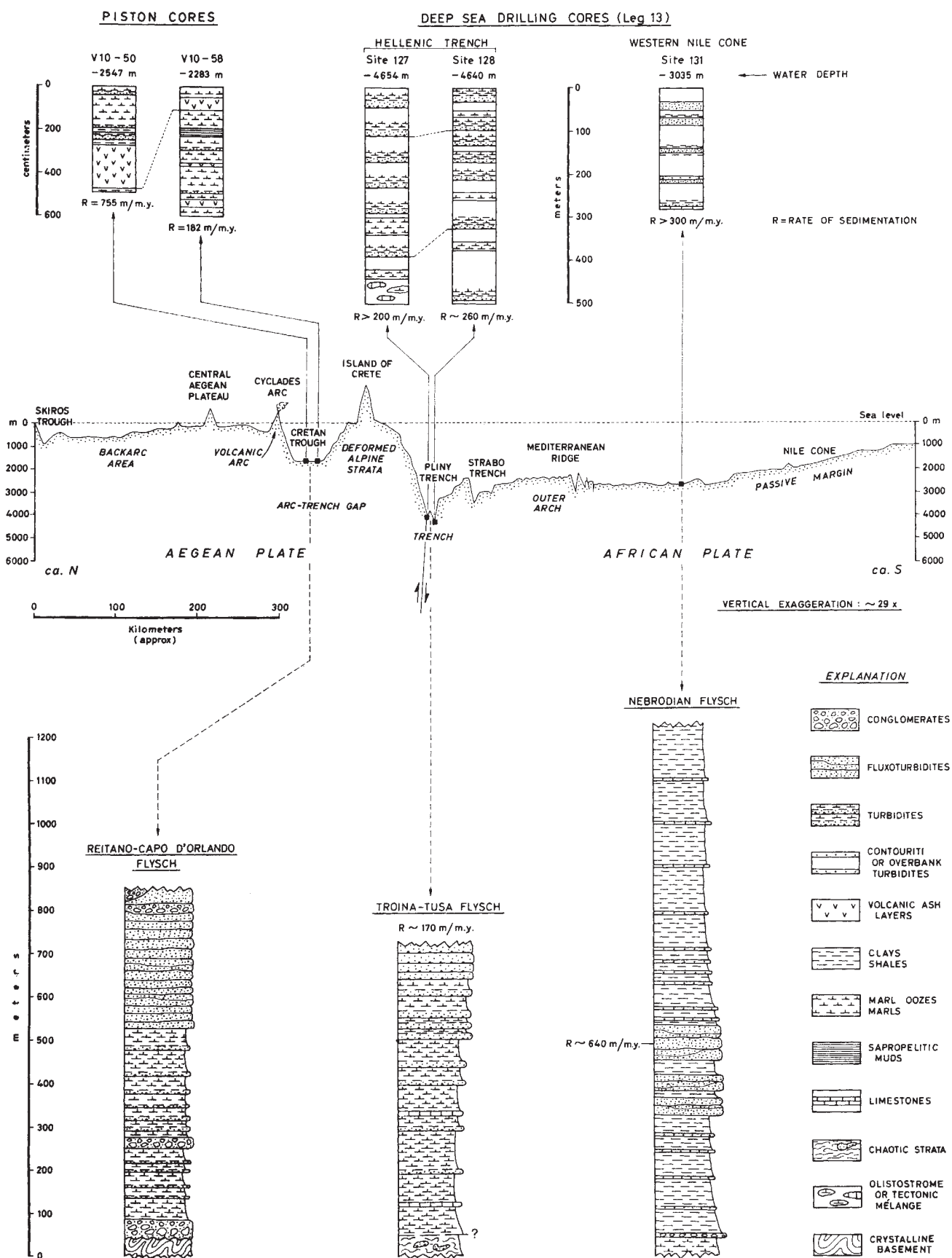


Fig. 1 The 'tectonic triad of flysch types' characteristic of a contracting basin, showing the present day (factual) and the ancient (inferred) tectonic settings for the three litho tectonic assemblages. In the upper part, the Quaternary sedimentary columns of the JOIDES cores⁹ and two Vema piston cores¹² were projected on to a bathymetric profile crossing the eastern Mediterranean and the southern Aegean at approximately 25°E¹³. The lower part shows generalised columnar sections of the three equivalent Oligo-Miocene flysch groups which crop out in the Sicilian part of the Maghrebian Chain.

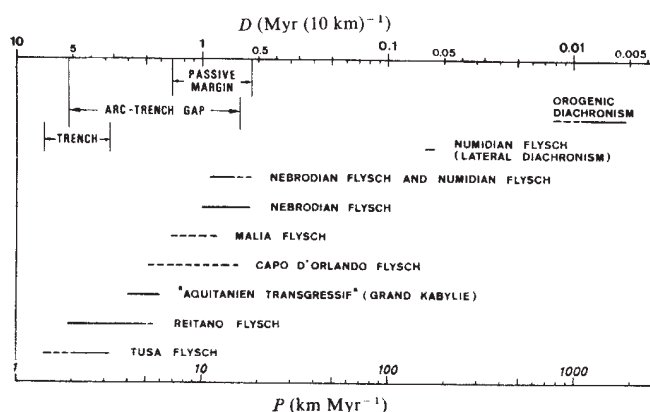


Fig. 2 Biostratigraphic estimates of the transgression of basal strata of the Oligo-Miocene flysch and of orogenesis in Sicily. Depositional events—trench (Tusa Flysch) and arc-trench gap sedimentation (Reitano-Capo d'Orlando Flysch)—are characterised by a greater diachronism than passive margin deposition (Nebroidian, Numidian and Malia Flysch). The migration of the crustal suturing along the Maghrebain Chain is near the boundary of the time resolution at present achieved with planktonic foraminiferal zonation. P , the rate of progression in km Myr^{-1} , and D , the diachronism coefficient in Myr (10 km)^{-1} , are new parameters proposed⁵ for a better quantitative evaluation of the magnitude of diachronism. Using these parameters 'bio-isochron lines' can be traced on geological maps, allowing estimations of relative past positions of passive and active continental margins.

data indicate a time span of only 3–4 Myr for a single 'tectogenetic cycle' which includes syntectogenetic trench sedimentation, tectonic deformation and the beginning of deposition of the arc-trench gap cover. In northern Morocco, between Tangiers and Ceuta, there is a spectacular juxtaposition of successive elongate thrust belts comprising Hellenic Trench-type flysch (Beni-Ider) and Numidian Flysch, and probably caused by the progressive accretion of deposits deformed in eastward facing subduction zones (F. Guerrero and W. B. F. Ryan, personal communication).

As in the case of the depositional events, the timing of orogeny is not synchronous everywhere in the Maghrebain Chain. Collision and suturing of the northern microplates and the North African continental margin occurred about 1 Myr earlier in Algeria¹⁰ (late Burdigalian) than in Sicily⁵ (early Langhian). The eastward migration of the locus of collision was accompanied by the termination of Numidian deposition. The most likely reason for this diachronous orogenesis seems to be either a non-parallelism between the approaching continental margins or an oblique closing of the flysch basin with some component of transcurent motion.

In view of these data it seems difficult to maintain the classical concept that the Maghrebain nappes originated primarily as a result of centrifugal gravitational sliding away from an uplifted Tyrrhenian zone¹¹. The noncontemporaneity of sedimentary and diastrophic events in different parts of the same orogenic belt also brings into question the validity of the rigid formalism of traditional (proto) stratigraphical philosophy (that is, the platonic idea of stratotypes). It seems that a dynamic (sedimentological ecological) approach would be more appropriate.

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Efficiencies of electrolytic and thermochemical hydrogen production

Two methods not involving fossil fuels for manufacturing hydrogen have been suggested as a basis for an eventual hydrogen economy: first, electrolysis of water using nuclear (or ultimately solar, geothermal or thermonuclear) electricity and, second, thermochemical cycles using nuclear heat, in general from a high temperature reactor^{2,3}. In the thermochemical processes, a series of chemical steps is envisaged that will allow the differential splitting of the H_2O molecule by means of reactions at different temperatures and with appropriate thermodynamic requirements to give high efficiency. The sequence of reactions, ideally of gas-solid type, acts as an adsorption-desorption-cycle heat engine. For economic hydrogen production, high thermal efficiency combined with low capital investment cost is necessary. The question is whether a system constructed on the basis of a sequence of chemical reactions will be more efficient (and ultimately less costly) than one based on a heat engine producing electricity, followed by electrolysis. Here I shall examine briefly some aspects of the efficiency of both approaches.

In the electrochemical case, the overall thermal efficiency is relatively simply defined as

$$e_t = \Delta H^0 / Q \quad (1)$$

where ΔH^0 is the standard heat of reaction for the oxidation of hydrogen to water under the conditions of use and Q is the total quantity of heat needed to effect the breakdown of 1 mol of water. If the electrolysis is reversible, that is, if the electrode processes (under conditions of no net reaction) with the reactants and products in their standard states have an exchange rate that is much higher than the desired net rate of breakdown, then, Q will be given by

$$Q = x\Delta G_t^0 + T\Delta S_t^0 \\ = x\Delta G_t^0 + (\Delta H_t^0 - \Delta G_t^0) \quad (2)$$

where ΔG_t^0 , ΔH_t^0 and ΔS_t^0 are the standard free energy, heat of formation and entropy of water at the temperature of electrolysis and x is the factor for the conversion of heat into (electrical) work. In general, liquid water will be used as feed-stock, therefore the ΔG_t^0 and ΔH_t^0 terms in Q will be those for liquid water. By contrast, the ΔH^0 term may be either for gaseous or liquid water, depending on whether or not condensation takes place during use of the heat.

In a real electrolyser, three types of loss occur, all of which add ΔG terms. The first results from the fact that the reactions at the electrodes have finite rates (overpotential). This is particularly so for the case of the oxygen electrode at normal temperatures. This overpotential is given by the Butler-Volmer equation in the most general case. The equation must, however,

be modified to take account of the effects of changing effective electrode area and of changing reactant concentration, which result from the remaining two losses—current distribution stemming from local resistance, and reactant depletion because of mass-transport effects (for example, the formation of product gas bubbles). In addition, irreversible energy loss occurs because of resistive (IR) drop throughout the system. Altogether, the additional free energy that must be provided to decompose water is conventionally represented by

$$\Delta(\Delta G_i) = \Delta G_{\eta} + \Delta G_{diff} + \Delta G_{IR} \quad (3)$$

where ΔG_{η} is the overpotential, calculated from the ratio of the net and exchange (equilibrium) rates of reaction using the Butler–Volmer equation, assuming the whole surface of the electrode to be active; ΔG_{diff} is the effective diffusion loss; and ΔG_{IR} is the loss attributable to the IR drop. For relatively small displacements from equilibrium, the ΔG_{η} and ΔG_{diff} terms linearise, so that the combined $\Delta(\Delta G)$ term is of pseudo-ohmic type. An expression for an uncomplicated single-electrode process under these conditions is given by

$$\Delta(\Delta G_i) = i \left[RT \left(\sum_n \frac{1}{i_{oj}} + \sum_m \left| \frac{1}{i_{dk}} \right| \right) + nFR' \right] \quad (4)$$

where i is the rate of decomposition (current per unit area), R is the gas constant, i_{oj} is the equilibrium reaction rate of the j th reaction step, i_{dk} is the diffusion-limited rate of the k th step, and R' is the internal electrical resistance per unit area. In general only one step in each case will predominate with regard to diffusional and kinetic limitations; however, if the limiting steps occur more than once in the overall process, the $1/i$ terms must be correspondingly summed over the number of times they occur.

In general, for electrolysis at low temperatures $\Delta G_i^0 + \Delta(\Delta G_i)$ will exceed ΔH_i^0 , since under these conditions kinetic and diffusional limitations are considerable and, in any case, the value of $\Delta H_i^0 - \Delta G_i^0$ is small (~ 12 kcalorie for liquid water at ambient temperature). All the energy required for water decomposition will thus be free energy in the form of electricity. At higher temperatures, ΔG_i^0 falls rapidly (ΔG_i^0 for water vapour ~ 44 kcalorie mol^{-1} at 700°C) and the kinetic and diffusional terms become less important, so that in principle only a part of the energy used need be electrical work, the rest being provided by heat.

For the thermal method of splitting water, which involves the use of a series of chemical cycles devised in such a way that the ΔG^0 in each reaction is close to zero, thermal efficiencies are much less easy to define in detail. In general, if one assumes a perfect system with infinitely rapid reactions (complete reversibility) with no heat losses, then the efficiency is given by an expression similar to equation (1), summed over all the n reaction steps to be used in the cycle, that is

$$e = \Delta H^0 / Q_{rev} = \Delta H^0 / [x \sum_n \Delta G_{i,j}^0 + \sum_n (\Delta H_{i,j}^0 - \Delta G_{i,j}^0)] \quad (5)$$

where the j e terms refer to the j th step⁴. If the reactions involve separations, particularly of gaseous components, an irreversible work of separation corresponding to $T\Delta S_m$, where ΔS_m is the entropy of mixing of the components, must be added to Q_{rev} (ref. 4). This is, of course, directly avoided in electrolyte procedures. For this reason, cycles with gas–solid reactions involving only one gas are to be preferred where possible. In addition, inevitable heat losses that cannot be partially used as work will occur in the processes. Hence Q is given by

$$Q_{rev} + x \sum T \Delta S_m + \text{heat losses}$$

So far in the literature, efficiencies for the thermal cycle

method of generating hydrogen have been calculated on the above basis. In several cases (see ref. 5) x , the factor for the conversion of heat into work, has even been ignored (see, however, refs 4 and 6).

All such calculations regard the processes as ideal—each step is not only at equilibrium but is infinitely rapid compared with the net rate of the reaction. This situation is obviously idealised and corresponds precisely to ignoring the overpotential factors in electrolysis. As in the latter, free energy losses occur because of the net rate of the processes resulting from three similar causes: kinetic, diffusional and frictional. The kinetic limitations of each step are given by the chemical equivalent of the Butler–Volmer equation, namely the Marcelin–De Donder equation⁷. This may be expressed in the form

$$k = k_0 [\exp(\vec{\Delta A}/RT) - (\overleftarrow{\Delta A}/RT)] \quad (6)$$

where k is the net rate of the process, k_0 is the equilibrium rate and ΔA , $\overleftarrow{\Delta A}$ are the displacements of chemical potential of the reactants and products from equilibrium (affinities) corresponding to overpotential in the electrochemical case.

In general, the reactions chosen for thermochemical cycles will be, in the optimum case, solid–gas reactions, in which the chemical potential of the reactants may be taken to be independent of the amount of material reacted. In these conditions, the measured (out of equilibrium) rate of reaction, on which process scaling is based, will be of the same order as that for the equilibrium rate. For a net rate close to this value, the minimum value of ΔA will be of the order of $1.3RT$ for each separate reaction step. Summed over all steps for a four-step process at an average temperature of 800 K , this represents 8.3 kcalorie of pumping work. Taking into account the x factor and system inefficiencies (viscous losses, concentration gradients), the total extra energy input may amount to 30 kcalorie per mol hydrogen as a reasonable minimum. Similar considerations apply to diffusion-limited steps, whether they occur in the chemical steps or in component separation. The latter will introduce additional work terms that must be added to the separation work. This extra energy can present a serious additional fall in overall efficiency. For example, a process with a reported estimated efficiency (hydrogen low heating value) of 45% (ref. 6), after all reversible work and heat losses are accounted for, will have a real maximum efficiency of only about 36% if one considers only the irreversible losses in the chemical steps. If the irreversible losses in separation steps are considered, the estimated maximum efficiency may approach 30% . This point illustrates the fact that irreversible thermodynamic considerations cannot be ignored in calculating the efficiencies of thermochemical cycles in which affinities must be summed over several steps, giving typical requirements for total extra work which may be greater than those for separation work. It seems, in fact, that, when all factors are taken into account, processes based on thermochemical conversion may prove to be less efficient than projected electrolyzers, both processes being supplied from a heat source at the same temperature.

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Enhancement in thermonuclear reaction rates in a dense plasma

FOR laser-driven fusion, a number density of the order 10^{26} cm^{-3} is usually envisaged^{1,2}; here I consider the effects of the presence of a medium on the nuclear reaction rates³ and show the feasibility of enhanced fusion rates when a certain condition is met. The results are applicable both to controlled thermonuclear reactions and to astrophysical conditions.

For a head-on collision of two particles carrying charges Z_1e and Z_2e in an electron-ion plasma with dielectric constant $\epsilon(\mathbf{k}, \omega)$, the Coulomb interaction is modified to⁴

$$V(\rho) = (4\pi Z_1 Z_2 e^2 / \nu) (2\pi)^{-3} \int d^3 \mathbf{k}_1 \int_{-\infty}^{\infty} d\omega \times \{ [k^2 \epsilon^2(\mathbf{k}, \omega)]_{k_z = \omega/\nu} \}^{-1} \exp \{ i(\omega \rho / \nu) \} \quad (1)$$

($Z_1 = Z_2 = 1$ for a deuterium-tritium (DT) mixture.) Here, ν is the velocity of the incident particle which is taken to be along the z direction. $\mathbf{k} = (k_1, k_2)$, and $\rho = z - \nu t$ is the distance between two colliding particles.

The ion velocity ν of interest usually far exceeds the ion thermal velocity $\nu_T = (3k_B T / M)^{1/2}$, where T is the ion temperature and M is the ion mass. (For a DT mixture, M is taken to be a suitably averaged ion mass.) Furthermore, when the electron temperature T_e exceeds T so that $\nu \ll u_T$ (where $u_T = (3k_B T_e / m)^{1/2}$ is the electron thermal velocity), we have

$$\epsilon(\mathbf{k}, \omega) \simeq (k_D / k)^2 + \{ 1 - [\Omega_p / (\omega + i\delta)]^2 \} \quad (2)$$

where $\delta \rightarrow 0+$, $k_D = (4\pi n_e e^2 / k_B T_e)^{1/2}$, and $\Omega_p = (4\pi n_e e^2 / M)^{1/2}$. (n_e is the number density of the electrons.) For $(k_D \rho)^2 \ll 1$, we have

$$V(\rho) \simeq (Z_1 Z_2 e^2 / \rho) \{ 1 - (k_D \rho) [\alpha \lambda(\alpha) + \theta(1 - \alpha^2)(1 - \alpha^2)^{1/2}] \} \quad (3)$$

Here

$$\lambda(\alpha) = (\pi/2) - \theta(1 - \alpha^2) \arctan[(1 - \alpha^2)^{1/2} / \alpha]$$

with $\theta(x)$ being the unit step function [$\theta(x) = 1$ for $x > 0$ and zero otherwise] and $\alpha = 3^{1/2}(\nu_s / \nu)$ where $\nu_s = (k_B T_e / M)^{1/2}$ is the ion sound velocity.

Since the factor $[\alpha \lambda(\alpha) + \theta(1 - \alpha^2)(1 - \alpha^2)^{1/2}]$ does not exceed $\pi/2$, the dynamic screening at a small distance may be ignored. But the second derivative of this factor with respect of ν is proportional to

$$\theta(1 - \alpha^2) / (1 - \alpha^2)^{1/2}$$

which becomes extremely large as $\alpha^2 \rightarrow 1-0$. [$\alpha = 1$ corresponds to the case where $\omega (= \nu k_z)$ is equal to the angular frequency of the ion-sound waves⁴, $\nu_s(3k_z)^{1/2}$.] To introduce a further simplification, I note that the reaction which starts the fusion is the one involving a head-on collision. So, I consider $V(\rho)$ given previously as the modified Coulomb interaction between the nuclei and the velocity ν in it as the relative velocity, ignoring the effects of the centre-of-mass (CM) motion, of two colliding nuclei in the plasma.

With this modified Coulomb repulsion, I obtain, using a standard method⁵, the thermonuclear reaction rate $r = n_1 n_2 \langle \sigma v \rangle$ (where n_1 and n_2 are the number densities of the colliding nuclei) as

$$\langle \sigma v \rangle \simeq \langle \sigma v \rangle_0 |g|^{-1/2} \exp \{ \epsilon_D [\alpha_0 \lambda(\alpha_0) + \theta(1 - \alpha_0^2)(1 - \alpha_0^2)^{1/2}] / k_B T \} \quad (4)$$

where the lowest order in the expansion parameter ϵ_D / E_0 (with $\epsilon_D = Z_1 Z_2 e^2 / k_B T$) is kept. Here,

$$\langle \sigma v \rangle_0 = 4(2/3\mu)^{1/2} S_0 (E_0)^{1/2} / k_B T \exp(-3E_0 / k_B T)$$

with

$$E_0^{3/2} = (\mu/2)^{1/2} (\pi Z_1 Z_2 e^2 k_B T / h)$$

and

$$\mu^{-1} = M_1^{-1} + M_2^{-1}$$

is the usual reaction rate per pair of nuclei for pure Coulomb repulsion. α_0 is $\nu_s (3\mu/2E_0)^{1/2}$ and S_0 is a constant. For a 50% DT mixture, we have $S_0 \simeq 1.3 \times 10^4$ barn keV (in the CM system). The exponential factor in the above equation is, in practice, close to 1 because $(\epsilon_D / k_B T) \ll 1$. So no dramatic enhancement is expected from this factor. The factor g represents a feasible enhancement and it is given by

$$g \simeq 1 - (\epsilon_D / 6E_0) \theta(1 - \alpha^2) [\alpha^2 / (1 - \alpha^2)^{1/2}] \quad (5)$$

where

$$\alpha = \nu_s (3\mu/2E_0)^{1/2}$$

with

$$E \simeq E_0 \{ 1 - (\epsilon_D / E_0) [(4/3)\alpha \lambda(\alpha) - \theta(1 - \alpha^2)(1 - \alpha^2)^{1/2}] \}$$

In general $g \simeq 1$ except when $\alpha^2 \rightarrow 1 - (\epsilon_D / 6E_0)^2$ or, equivalently, T_e exceeds T such that

$$T_e / T \simeq (2M/3\mu)(3E_0 / k_B T) [1 - (2/3)(\hbar \Omega_p / k_B T)] \quad (6)$$

When the conditions implied in equation (6) are met, $g \rightarrow 0$ which gives the desired anomalous increase in $\langle \sigma v \rangle$. (For a superdense plasma where $T_e \ll T_F$, T_e in equation (6) should be replaced by $2T_F/3$. Here T_F is the Fermi temperature of the degenerate electrons.) For a 50% DT mixture, equation (6) becomes

$$T_e / T \simeq (28/T^{1/3}) [1 - 3.7 \times 10^{-3} (n_e / T)^{1/2}] \quad (7)$$

(The units of n_e and T are 10^{26} cm^{-3} and keV respectively.) For example, for $T = 5$ keV, $T_e \simeq 82$ keV is required to satisfy equation (7). Since the bremsstrahlung losses³ are proportional to $T_e^{1/2}$, an additional loss is incurred when $T_e \gg T$ compared with the case when $T_e = T$. And one requires $|g| < (T/T_e)$ (or $|g| < 6.1 \times 10^{-2}$ in the case of a 50% DT mixture) to offset the losses.

In the case when $T_e \ll T$ so that $\nu \gg u_T$ as well as $\nu \gg \nu_T$, we have⁴

$$\epsilon(\mathbf{k}, \omega) = 1 - [\omega_p / (\omega + i\delta)]^2, \quad (8)$$

where $\omega_p = (4\pi n_e e^2 / m)^{1/2}$. In this case, we obtain

$$\langle \sigma v \rangle \simeq \langle \sigma v \rangle_0 [1 + (\hbar \omega_p / 6k_B T)] \times \exp \{ (3E_0 / k_B T) (\hbar \omega_p / 6k_B T) \} \quad (9)$$

Thus, for a superdense plasma, a huge enhancement is expected from the exponential factor. But at such a high density T_F exceeds T , and T_e should be replaced by $2T_F/3$ in μ_T when $T_e \ll T_F$. So in order to have a significant enhancement, we should also require $T \gg T_F$ which gives a limit on n_e as

$$n_e \ll (3\pi^2)^{-1} (2mk_B T / \hbar^2)^{3/2}$$

[or $n_e \ll 1.4 \times 10^{26} T^{3/2} \text{ cm}^{-3}$ (T in keV) for a 50% DT mixture]. I note that even at $n_e = 10^{26} \text{ cm}^{-3}$, $\langle \sigma v \rangle / \langle \sigma v \rangle_0$ is only about 1.2 for a 50% DT mixture at $T = 5$ keV. Thus, one finds no significant increase in $\langle \sigma v \rangle$ when $T_e \ll T$.

In conclusion, dynamic screening has a negligible effect in most circumstances, except when T_e exceeds T by the amount

given by equation (6). In this case, an anomalous enhancement in thermonuclear reaction rates occurs.

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Garden hose separation of gaseous isotopes

METHODS of separation of isotopes and in particular the separation of uranium isotopes, have long been studied^{1,2}. Here I present a new variation of the "time-of-flight" process, applicable to all gaseous isotopes, although UF_6 gas is considered as the example.

The time-of-flight separation is basically a mechanical analogue of a diffusion barrier. As a cloud of gas moves through an evacuated region, the faster moving lighter molecules concentrate toward the outer portion of the beam configuration, and when they are collected they are enriched in the light component. Similarly, the inner portion would be depleted in the light component when collected. But a continuous and stationary source beam would not give rise to separation as the inner portion of one layer of cloud is equivalent to the outer portion of the subsequent layer of cloud. A 'chopper' is therefore required to cut the beam into individual layers, and extensive pumping is needed to evacuate the system sufficiently to maintain the integrity of the cloud during their time of flight.

During World War II, Bagge designed such an apparatus³; with synchronised choppers, collectors, and powerful pumps, a cascade of such time-of-flight units were constructed to separate the uranium isotope. But the necessity of operating that system at very low gas pressure and with limited efficiency of collection are major drawbacks implying low throughput and high consumption of pumping energy.

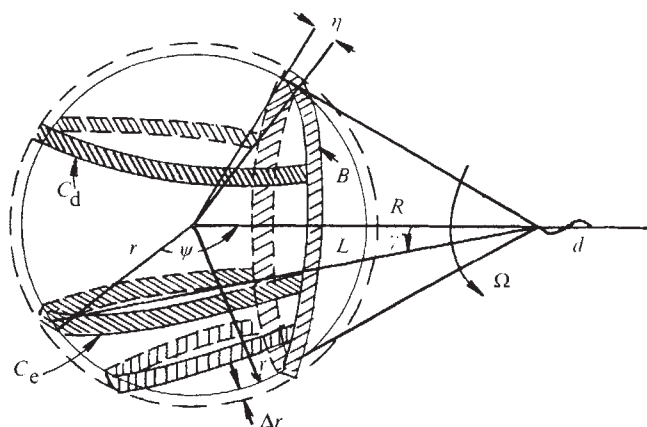


Fig. 1 Schematic relationship between the population fronts and the collectors B, Ce, and Ca, where η is the angular span of collector B. B and Ce are for enriched light component, while Ca is for depleted light component. Δr is the mean separation of the population fronts.

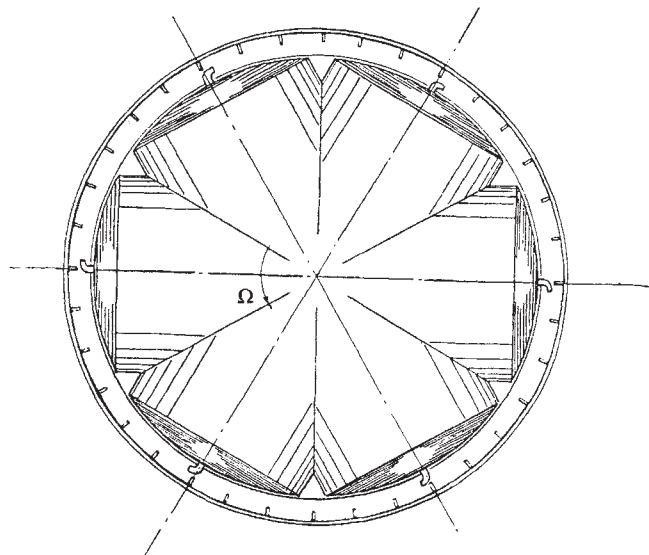


Fig. 2 A diagrammatic plan view of the centrifugal separating chamber with three circular compartments at the wall storing the enriched, depleted, and to-be-recycled concentrations. For $v_e \approx 2v_0$, there are six ejection outlets symmetrically disposed in a plane of rotation, with each outlet standing for three separate outlets stacking on top of each other for their corresponding storage chambers.

In the technique I propose, the gas molecules are channelled into an evacuated chamber through a set of rotating supersonic nozzles at a small retarding angle so that the ejected gas has zero angular momentum. Once inside the vacuum chamber, molecules travel according to their corresponding thermal velocities plus a common ejection velocity v_e , and from

$$mv_0^2/2 = 4kT/\pi = \text{constant} \quad (1)$$

the difference in mass gives,

$$\Delta v_0 = -\Delta mv_0/2m = -(2kT/\pi m)^{1/2} \Delta m/m \quad (2)$$

From the Maxwellian equal partition relationship, we have $\Delta v/v = -\Delta m/2m$ in general. After time t , while the ejection velocity carries the molecules to a distance $R = v_e t$ from the nozzle, the thermal velocity would carry them into spherical fronts of radius r centred at the end point of R , and the separation of the fronts Δr is

$$\Delta r = \Delta v t = -\Delta m v R / 2m v_e = 0.426 \% \text{ of } v R / v_e \quad (3)$$

for UF_6 .

The molecular fronts are now in the form of a ball (thermal velocities) on top of a cone (ejecting velocity), so the objective is to design rotating collectors to skim the surface of the ball most effectively in order to collect a substantial portion of the concentrations separated by the two sides of the ball surfaces. Figure 1 is a schematic of collectors C_e (for the enriched front), C_d (for the depleted front) and B (for the relatively stationary collector of the original Becker design); the angular width of collector B can be shown as $\approx 2 \cos^{-1} (1 - \Delta r/r) \approx 10^\circ$.

Once the molecule enters the collector, it would be swung toward the chamber wall by the centrifugal force, and it is stored and pressurised by the rotating wall outside the chamber. For $v_e = 2v_0$, there can be as many as six sets of nozzle-collector combinations operating at the same plane (Fig. 2). The storage chamber has three compartments for enriched, depleted, and to-be-recycled concentrations. The last part is

mainly the 'unskimmed' portion which is channelled back to the original nozzles that ejected them. There are no moving parts relative to the chamber in this collector-nozzle-chamber combination, and by operating under vacuum (~ 0.1 torr), UF_6 can be stored in its solid form so that the flow can be controlled by thermal evaporation as well.

Following the Maxwellian distribution, the normalised population density $n(v)$ is,

$$n(v) = (m/2\pi kT)^{3/2} 4\pi v^2 \exp(-mv^2/2kT) \quad (4)$$

where

$$\int_0^\infty n(v) dv = 1$$

and

$$\int_0^\infty n(v) v dv = (8kT/\pi m)^{1/2}$$

Consider first the high energy Maxwellian tail, for $v' \geq v_0$, let $v^2 \equiv v'^2 + u$, $2kT/m$ and $g \equiv mv'^2/2kT$, so the fractional population at the tail portion is

$$\begin{aligned} n(v \geq v') &= \int_{v'}^\infty n(v) dv = (2/\sqrt{\pi}) e^{-g} \int_0^\infty (u+g)^{1/2} e^{-u} du \\ &= (2/\sqrt{\pi}) e^{-g} [g^{1/2} + 1/2 g^{-1/2} - 1/4 g^{3/2} + \dots] \end{aligned} \quad (5)$$

and calculate the increment Δn by differentiations of $n(v)$ and integrate,

$$\Delta n(v \geq v') = (-4/\sqrt{\pi}) e^{-g} g^{3/2} \Delta v'/v' \quad (6)$$

then

$$\Delta n/n \simeq [-8g^3/(4g^2+2g-1)] \Delta v'/v' \quad (7)$$

Similarly, at the low energy part of the spectrum where $g \ll 1$,

$$n(v \leq v') \simeq (4/\sqrt{\pi}) g^{3/2} (1/3 - g/5 + g^2/14) \quad (8)$$

and

$$\Delta n/n \simeq e^{-g} (\Delta v'/v') (1/3 - g/5 + g^2/14) \quad (9)$$

Figure 3 shows plots of equations (5)–(9). Clearly, concentrating on the high energy tail alone, α can be made as large as that of total separation, only at the expense of reducing the total fractional population by the Boltzmann factor e^{-g} . The depleted fractional from equation (9) has a relatively stable separation at $\Delta n/n \simeq 1\%$.

I now make a simple estimate of the specific power requirement of the 'gross-cut-mode' from equations (7) and (9). Let y be the enriched stream, x the depleted stream, θ the cut of separation, then $y = \theta(1 + \Delta n/n)$, $x = (1 - \theta)(1 - \Delta n/n)$, and from $\alpha \equiv (y/1 - y)/(x/1 - x)$, ignoring terms with $(\Delta n/n)^2$, we have

$$\alpha \simeq \theta(\theta + \Delta n/n)/[(1 - \theta)(1 - \theta - \Delta n/n)] \quad (10)$$

Choose $|\Delta n/n| \simeq 1\%$ for both x and y , so from Fig. 3,

$$n(v \geq v' \text{ at } g \simeq 2) \simeq 25\% \simeq n(v \leq v' \text{ at } g \simeq 0.7)$$

and let the collectors catch one-half of the designated fractionals, so one-quarter of the population is processed while

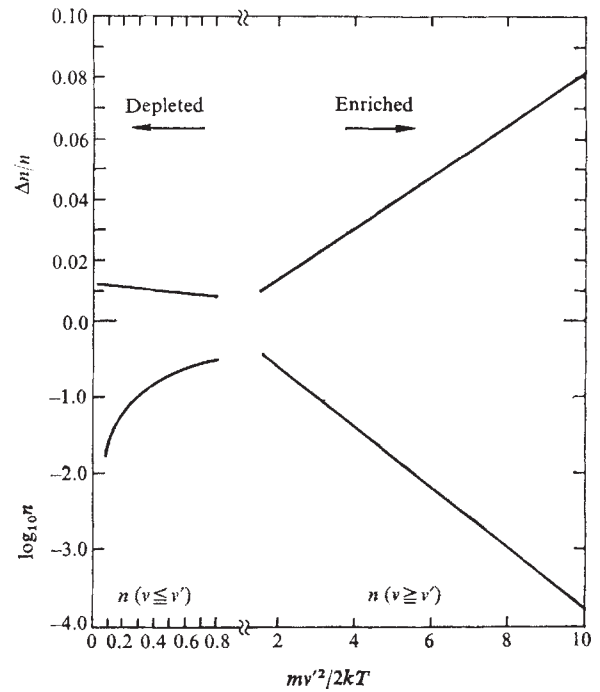


Fig. 3 Concentrating on the high energy tail of the Maxwellian distribution, $\alpha \simeq 1 + 4\Delta n/n$ can be made large at the expense of reducing fractional population collected. At the low energy end, $\Delta n/n$ is relatively stable at 1%. A crude estimate of processing a quarter of the population at each end of the spectrum gives a specific power consumption only one-fifth that of the minimum theoretical diffusion requirement.

three-quarters is channelled back to the nozzles to be recycled. With $\theta = 1/2$, from equation (10),

$$\alpha \simeq 1 + 4\Delta n/n \simeq 1.04 \quad (11)$$

Let $R = 100$ cm, $r = 50$ cm, and the height of the chamber $D \simeq R + r = 150$ cm, $\Omega = 2\pi \times 30$ Hz so the velocity of the collector $v_c \simeq \Omega R \simeq 2v_0$ at room temperature. The mean free path $\lambda \simeq D \simeq 150$ cm so the pressure $P_0 = n_0 kT \simeq 1.75 \times 10^{-2}$ dyne cm^{-2} with molecular diameter $\simeq 6$ Å. Because of the centrifugal pumping, once the molecules are caught by the collectors, the gas pressure is raised by the factor $mv_c^2/2kT = 5.8$ so pressure in the collector $P_c \simeq 0.1$ dyne cm^{-2} . The number of molecules N flowing through the processing chamber per second is

$$\begin{aligned} N &= 6 \times v_c \times n_0 \times \pi(D/2)^2 \\ &= 1.2 \times 10^{21} \\ &= 0.7 \text{ g of UF}_6 \\ &= 15 \text{ t of uranium per year.} \end{aligned} \quad (12)$$

The separating collectors carry only a quarter of this value, so the throughput is $15 \times 1/4 = 3.75$ t yr^{-1} . The power requirement for the collectors and the shields acting as a centrifugal pump is

$$0.5 mv_c^2 \text{ s}^{-1} = 28 \text{ W} \quad (13)$$

By balancing the compression energy of the gas with that of the jet flow of $0.5 mv_c^2$ at 25 W, the nozzle opening should be about 0.5 cm and from the continuity of flow, $P_{\text{nozzle}} = P_0 \times (150/0.5)^4 = 9 \times 10^4 P_0$, so the additional compressional power required is

$$(m/M) RT \log(P_{\text{nozzle}}/5.8P_0) \simeq 20.7 \text{ W}, \quad (14)$$

where R is the gas constant, M the molecular weight of UF_6 and

m the mass flow in g s^{-1} . The Unit-of-Separative-Work (USW) done for each stage is now,

$$\begin{aligned}\text{USW} &= \text{Throughput} \times (\alpha - 1)^2 / 2 \\ &= 3.75 \times 10^3 \text{ kg yr}^{-1} \times 0.04^2 / 2 \\ &= 3.0 \text{ kg yr}^{-1}\end{aligned}\quad (15)$$

and the specific power requirement is,

$$\frac{1}{3} (0.028 + 0.0207) = 0.0162 \text{ kW}/(\text{kg USW}) \text{ yr}^{-1}, \quad (16)$$

which is only 22% of the theoretical minimum requirement of the diffusion process ($0.073 \text{ kW}/(\text{kg USW}) \text{ yr}^{-1}$). The throughput of this method is relatively low so the specific capital cost may be higher. On the other hand, by using UF_6 in a solid form for storage, together with a much higher separation coefficient, the number of processing stages required can now be reduced by over an order of magnitude. The present method also compares favourably with a centrifuge in terms of centrifugal force requirement and the choice of variable separation constant α .

Some of the recent methods of multistage preferential heating of isotopes by lasers can also be incorporated into the present method, as the time-of-flight vacuum chamber preserves the original state of motion as well as that added and the chamber dimension near the axis of rotation is such that it can easily accommodate these additional arrangements.

I thank Dr M. Benedict of MIT for showing me related literature, my colleagues in Combustion Engineering for comments and Mr A. Davis for drawing Fig. 2.

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Radium removal from drinking water

RADIUM is absorbed from natural waters on to acrylic fibres impregnated with oxides of manganese¹. This extraction technique, developed for oceanographic studies, is also effective in removing radium from drinking water.

Radium-226, a naturally occurring radioactive daughter of ²³⁸U, presents a potential health hazard when dissolved in ground waters. The body metabolises radium and calcium similarly; if radium is available to the bloodstream, it will concentrate in the bones². Radioactive decay of ²²⁶Ra within the bones exposes the skeleton to highly ionising, short range alpha particles as well as more penetrating beta and gamma radiation from radium daughters. The most immediate effect of this radiation is to increase the chances of developing osteosarcoma and other cancers². Although the exact relationship between the low level radium exposure and cancer risk is not known, there is probably no safe level of human exposure to radium. The drinking water standards published by the United States Public Health Service recommend³ a level not exceeding 3 pCi l^{-1} ($6.66 \text{ d.p.m. l}^{-1}$).

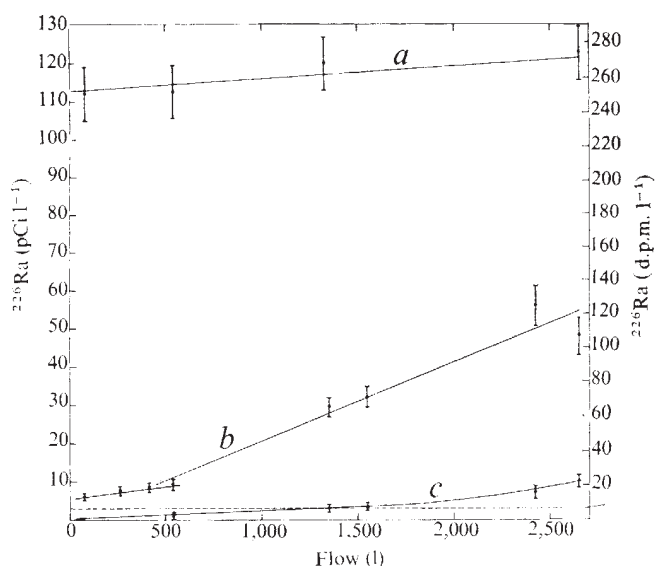


Fig. 1 Concentrations of ²²⁶Ra measured in the water from the well and in the effluents from two columns filled with manganese-fibre. The first 500 l were processed at 4.3 to 6.2 l min^{-1} , the remainder at 7.5 to 8.2 l min^{-1} . The variable slopes of the first column effluent reflect this difference in flow rate. a, Well effluent; b, first column effluent; c, second column effluent. - - - -, US Public Health Service drinking water limit.

Commercial grade uranium deposits in south Texas have been mined since the late 1950s. In 1971, elevated ²²⁶Ra levels were measured in well water near the edge of the Felder uranium ore body near Three Rivers, Texas⁴. Other wells in the south Texas area have been surveyed and a small fraction produce drinking water with radium levels above the United States Public Health Service Standard⁵. One of these wells, referred to as Whitley well No. 1, is located about 8 km south-west of George West, Texas. This well has a ²²⁶Ra concentration of 110 pCi l^{-1} , the second highest level measured in recent surveys of this area⁴. Small deposits of uranium occur within 300 m of this well; a high grade uranium deposit is being mined within 8 km. These localised ore bodies are believed to be the sources of radium found in the well waters of south Texas. Wells known to produce water high in ²²⁶Ra have been removed from use as routine drinking water sources.

Acrylic fibre impregnated with manganese has been used to remove radium from seawater. We prepared similar fibre using the procedure described by Moore and Reid¹. The resulting fibre contained 12–15% Mn by weight when dry.

Two water filtering columns connected in series were each filled with 40 g of Mn fibre. The columns were attached to Whitley well No. 1. A water meter attached downstream of the columns recorded the volume of water processed. Water samples were periodically drawn (a) directly from the well, (b) from the column 1 effluent, and (c) from the column 2 effluent. These samples were stored for ²²⁶Ra determinations using the ²²²Ra emanation technique⁶.

Initially the flow rate decreased slightly due to packing of the Mn fibre. By adjusting the water pressure we attained a steady flow of 7.5 to 8.2 l min^{-1} after about 550 l had passed through the system. The experiment was terminated after 2,650 l had been processed.

Our objective was to determine if radium could be effectively and inexpensively removed from this well water. Figure 1 shows that radium removal from this highly contaminated well by two Mn-fibre columns was essentially complete for 1,300 l. After 1,300 l had passed through the system, the column 2 effluent was 3 pCi l^{-1} , the Public

Health Service recommended drinking water limit. After 2,650 l of water had been processed, the outflow from column 2 still had less than 10% of the ^{226}Ra concentration of water entering the system. We conclude that radium removal from highly contaminated ground waters is technically feasible using manganese impregnated fibres. Although this experiment represents only one well, our success with this water as well as similar success with seawater and brackish water which contain high concentrations of calcium and magnesium¹, makes us optimistic about the general applicability of the Mn-fibre technique for removing radium from drinking waters.

The cost of removing radium using the Mn fibres is low. The fibre can be prepared for about \$15.00 per kg. One kilogramme fibre would bring the radium level of more than 10,000 l of the water from Whitley well No. 1 to within the United States Public Health Service limit. This would be adequate drinking water for a family of four for 3 yr (ref. 7). Since most wells in the area have ^{226}Ra levels much below that of Whitley well No. 1, the 10,000 l figure is a minimum. We conclude that manganese fibres can provide an inexpensive means of removing radium from drinking water.

We emphasise that this work is still in the testing stage. Any attempts to decontaminate drinking waters with respect to radium should be conducted only under the supervision of local health authorities.

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Methylation of lead in the environment

LEAD is one of the most toxic metals found in the environment and is of great concern because of its widespread occurrence in nature, but its fate is largely unknown¹. Much of the lead dispersed by man is eventually washed into natural waters and is presumably precipitated into the sediments². The methylation of mercury and arsenic by microorganisms in the environment has been documented and well summarised³. Nothing, however, has been known about the existence of organic forms of lead in the environment as a result of biotransformation⁴.

Here we present evidence for the first time, that microorganisms in lake sediments can transform certain inorganic and organic lead compounds into a volatile tetramethyl lead (Me_4Pb). Experiments were carried out with 50 g of sediment and 150 ml of lake water from Hamilton harbour (Lake Ontario), Mitchell Bay (Lake St Clair), and Erieau harbour (Lake Erie), placed individually in a

250 ml filtering flask fitted with a side-arm. Nutrient broth (0.5%) and glucose (0.1%) were added to stimulate the microbial growth and the flask was capped and sealed. Nitrogen was bubbled in to create an anaerobic condition but this practice was later discontinued because the microbial growth was sufficient to generate the anaerobic condition. After incubation for 2 weeks at 20° C, the gas phase in the incubation flasks was withdrawn through the side-arm by means of a peristaltic pump and transferred to a U-tube containing 3% OV-1 at -70° C. The sample trapped in the U-tube was swept into a gas chromatograph-atomic absorption spectrophotometer system for the separation and analysis of the volatile lead compound. Tetramethyl lead was detected in the air sample with reference to the retention time of a synthetic compound. The identity of the Me_4Pb peak in the air sample was further confirmed by gas chromatography and mass spectrometry. Addition of inorganic lead nitrate or organic trimethyl lead acetate (Me_3PbOAc) at 5 mg (expressed as lead) per litre of sample greatly increased the Me_4Pb production. Not all sediment samples we examined, however, produced Me_4Pb in identical conditions to those described above. In some cases, the addition of inorganic lead nitrate or chloride failed to affect the transformation but in all cases the transformation of Me_3PbOAc to Me_4Pb was observed. There was no transformation if the systems were autoclaved. Ultraviolet light irradiation caused no conversion of Me_3PbOAc to Me_4Pb in the absence of microorganisms, so ruling out the possibility of chemical disproportionation reactions⁵, activated by ultraviolet light.

Direct chemical synthesis of methyl lead compounds through alkylation of inorganic lead salts is very difficult because of the extreme instability of the postulated first intermediate monomethyl lead salts. We have observed the conversion of lead nitrate and lead chloride to Me_4Pb on several occasions in lake sediments but no Me_4Pb transformations have yet been detected from lead hydroxide, lead cyanide, lead oxide, lead bromide, or lead palmitate. In none of these experiments were Me_3Pb^+ salts detected in the culture medium, although it is a stable intermediate in the chemical pathway of the successive methylation. (The analysis for trimethyl lead salts was specially developed in our laboratories in support of this study.)

The pathways for the biological conversion of lead are not well understood. It is apparent that the conversion of inorganic lead to organic lead is a difficult process and it probably requires specific physical, chemical, as well as biological conditions. On the other hand, the biological methylation from Me_3Pb^+ to Me_4Pb seems to proceed quite readily and we were able to demonstrate this conversion with pure species of bacterial isolates from Lake Ontario without the presence of sediment. We found that species of *Pseudomonas*, *Alcaligenes*, *Acinetobacter*, *Flavobacterium*, and *Aeromonas* growing in the presence of nutrient broth (0.5%), glucose (0.1%) and yeast extract (0.1%) could transform Me_3PbOAc into Me_4Pb . None of the isolates was able to produce Me_4Pb from inorganic lead in chemically defined media.

Weekly sampling of the atmosphere in the incubation flasks was carried out to determine the rate of conversion of Me_3PbOAc to Me_4Pb . With 10 mg of lead (as Me_3PbOAc) in the sample, 125 μg of lead (as Me_4Pb) was detected after incubation for 1 week. The conversion rate increased to 642 μg in the second week and declined to 550 μg and 256 μg of lead in the third and fourth weeks respectively. The decrease in lead conversion after 2 weeks could be due to the exhaustion of nutrients, accumulation of toxic metabolic products, or changes in pH in the medium. Assuming that 642 μg of lead is the maximum rate for the conversion, the microorganisms were able to convert 6% of lead in one week.

The toxicity of Me_4Pb towards algae was studied by

bubbling the biologically generated Me_4Pb from one flask (containing 5 mg Pb l^{-1} as Me_3PbOAc) into the culture medium in another flask where a test alga *Scenedesmus quadricauda* was grown. As Me_4Pb is not soluble in water and is volatile, the exposure of an alga to this lead compound was only momentary. We estimated that less than $0.5 \text{ mg Pb}(\text{Me}_4\text{Pb})$ had passed through the culture medium. We found that the primary productivity and cell growth (determined by dry weight), however, decreased by 85% and 32% respectively, as compared with the controls without exposure to Me_4Pb . Furthermore, cells exposed to Me_4Pb tended to clump together. Similar results were obtained with *Ankistrodesmus falcatus*. To obtain similar inhibition, twice as much lead, in the form of Me_3PbOAc , and twenty times as much lead nitrate would be required. These numbers were calculated from our studies on the toxicity of various forms of lead compounds on algae.

We conclude from the results of 50 experiments that incubation of some lead containing sediments generates Me_4Pb ; that Me_3Pb^+ salts are readily converted to Me_4Pb by microorganisms in lake water or nutrient medium, with or without the sediment, and in the presence or the absence of light; that conversion of inorganic lead (such as lead nitrate or lead chloride) to Me_4Pb occurred on several occasions in the presence of certain sediments; and that the conversion is purely a biological process.

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A study of age group track and field records to relate age and running speed

AGE affects man's ability to run fast but to date there has been little interest in quantitating the relationship. The few published studies, such as the one by Dill on marathon runner Clarence DeMar¹, are concerned mainly with reporting lung volume, heart rate, and other physiological characteristics of notable performers, with only brief reviews of race performances over the runner's life span.

Two recent compilations of track and field records present an opportunity to study ageing effects on running speed. One contains records for men², in 1-yr age groups for ages 1 to 78, and the other contains women's records³, in 1-yr age groups for ages 3 to 60. This study was designed to determine how rapidly running speed deteriorates with age and whether the rate of deterioration depends on the length of the race; and to compare deterioration rates of running speed, strength and stamina in men and women.

When speed (m s^{-1}) is plotted against age, it is evident that speed improves up to age 20 or so and gradually deteriorates beyond age 30. Between ages 20 and 30, running speed is near the maximum and almost constant for all distances included in the records (100 m, 200 m, 400 m, 800 m, 1,500 m, 3,000 m, 5,000 m, 10,000 m and marathon (42,195 m)). An exponential model was chosen to fit the age records for each distance:

$$Y = A_1 [1 - \exp(A_2 X)] + A_3 [1 - \exp(A_4 X)],$$

where Y is the speed in m s^{-1} , X is the age in years, and the constants A_1 , A_2 , A_4 , A_3 are determined from the age records by the method of least-squares. This model has the advantage of fitting the data well over the entire age range, whereas the more usual Gompertz-type equations can be applied only to ages where speed is decreasing with age. In addition, this model allows the rate of increase in speed (under age 20) to differ from the rate of decrease (over age 30). The model also goes through the origin so that at zero age the speed is zero. Examples of the fit for several running events are shown in Fig. 1.

A comparison of the fit curves for all the men's running events reveals that age of maximum performance increases with distance. This is also true of the recorded data, where the sprint records are held by men in their early twenties but the marathon record-holder is 26 yr old. Comparison of the slopes of the fit curves reveals that speed deteriorates more slowly at longer distances than at shorter ones. For example, at age 50 speed in the 200 m sprint is slowing at a rate of $0.09 \text{ m s}^{-1} \text{ yr}^{-1}$ but for the marathon the rate is only $0.06 \text{ m s}^{-1} \text{ yr}^{-1}$. This observation suggests that strength deteriorates faster than stamina.

This hypothesis was tested further by fitting the exponential model to the age records for the men's shot-put and discus, events which are even more strength-dependent than the 200 m sprint. Direct comparison of the slopes for running events with those for the field events is not valid because the recording units differ. A comparison can, however, be made if all age records are converted to percentages of the world record.

Fig. 1 Running speed records by age. Exponential curves (for model, see text) were fitted to published age records. (a) Men's records for 200 m, 800 m, and marathon (42,195 m). (b) The 400 m records for men and women.

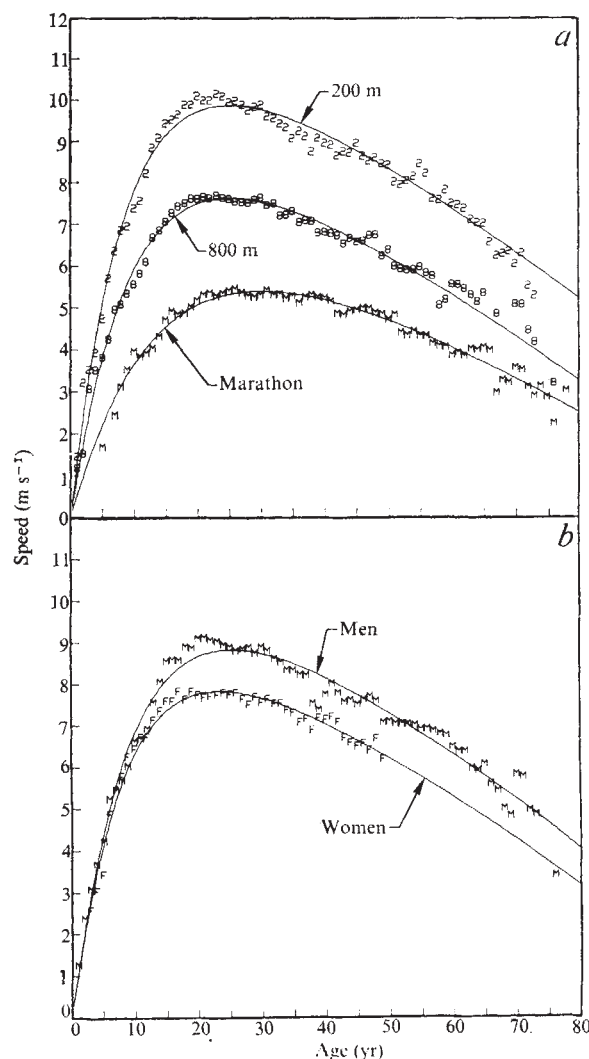


Table 1 Age records as percentages of world records

Age	Shot-put	Discus	200 m	Marathon
10	61	60	79	68
20	91	90	98	94
30	100	100	99	100
40	98	95	93	96
50	76	78	84	88
60	49	53	74	76

Percentages based on fitting the exponential model (see text), to published data.

Thus, a speed of 8.12 m s^{-1} for the 200 m race is 80% of the world record speed (10.15 m s^{-1}), whereas a shot-put of 17.46 m is 80% of the corresponding world record (21.82 m). These comparisons (Table 1) lend further support to the hypothesis that strength deteriorates faster with age than does stamina.

Fewer data are available on women's age records (current records only include running events up to 3,000 m, and rarely are there records for women over 45 yr of age). A comparison with those for men suggests that girls mature faster than boys (with respect to running events) in the age range 8–12 yr. For example, at ages 8, 9 and 11 women's records are faster than those for males in the 400 m run (see Fig. 1b). It also appears that beyond age 30, speed deteriorates at a faster rate for women than for men. For example, the fit curves show that in the 100 m sprint the women's record at age 45 is 85% of the women's world record, whereas the corresponding figure for men is 90% of the men's world record.

The records analysed here comprise the marks of many individual athletes but they can be thought of as those set by a 'super' runner; one who is in top condition throughout his life span. The ordinary runner is one whose speed is slower, perhaps by some fixed amount over the entire age span, than that of the super runner. The speed curve for the ordinary runner may have the same shape as that of the super runner and the rates of change of speed with age would be the same. This hypothesis could be tested by following up several runners over their life spans, recording annually their running times over measured distances. This could be done retrospectively, by contacting those who have run for a number of years and determining their best times at various distances each year.

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The capacity for joint visual attention in the infant

LITTLE is known about how visual attention of the mother-infant pair is directed jointly to objects and events in the visual surround during the first year of the child's life. To what extent does the child follow the mother's lead and the mother the child's, and what are the processes involved? The ability of the infant to respond successfully to such signals allows the mother to isolate and highlight a much wider range of environmental features than if the infant ignores her attention-directing efforts. We report a preliminary investigation of the extent of the infant's ability to follow changes in adult gaze direction during the first year of life.

Mothers who had volunteered as subjects in response to

newspaper advertisements were asked to bring their infants into our laboratory at a time when the infant was 'usually active'. Thirty-four infants, 2–14 months old, were tested in a small, sparsely-furnished room (15 ft by 10 ft) with a one-way screen at one end and a window with drawn blinds at the other. The infant was placed in a highchair, appropriate to his age, in an upright position. The mother played with him until he seemed settled and was then replaced by the experimenter—either male or female in their twenties, unknown to the baby. It was not practicable to have the mothers as experimenters with the rather strict testing requirements used here although many observations were made prior to this on other mothers and infants in a similar, though less controlled, situation. The experimenter first played with the infant for a short period and, if the latter showed no signs of distress, the mother left the room. The experimenter remained seated in front of the infant, eyes at the same level, about 0.5 m away. The infant was then given two trials in a prearranged order.

On each trial the experimenter first made eye-to-eye contact then silently turned his (her) head through 90° to fixate a small (concealed) signal light, 1.5 m away, for 7 s. He then turned back to interact with the infant. Two trials were given, one involving a head turn to the right, one a turn to the left, with inter-trial intervals varying from 20 to 50 s, depending on difficulties in establishing subsequent eye-to-eye contact. The infant's behaviour was recorded by two concealed video cameras set at 45° to the experimenter-infant face-to-face axis, giving a split-screen 45° profile of the infant from two sides. The experimenter was not visible on the screen. The infants were also filmed for calibration looking at experimenter in fixed positions of known angular displacement.

Trials were scored from the videorecord by the experimenter, based on infant head movement only. A positive response was scored if the infant looked (a) in the same direction (right/left) but not down at the floor or up at the ceiling; (b) without an intervening look elsewhere (ignoring short looks down during postural adjustment); (c) within 7 s; and (d) appeared to be looking for or at something (involving halting the head turn for 0.5 s or more with a cessation of limb movement). The infant did not have to appear to be fixating exactly the same point. Scoring reliability was ascertained from three naive observers uninformed as to experimenter's judgment. They produced 96% agreement on trials scored as positive, 90% on those scored as negative.

The proportion of infants judged as having produced a positive response on one or both trials increases steadily with age (Table 1). The form of the response, that is, how soon and where the infants look, did not show systematic change with age. Latencies in response were very variable—any point up to the end of the trial, and the infants would look anywhere from about 20° to 90° away from the midline. Labelling responses as positive does not, of course, mean that infants were looking for something to look at. In the upper age groups, however, there was strong evidence to suggest this may be so with infants often looking away, looking back at the experimenter and then looking away again. This had been even more marked when the mother was the experimenter in earlier observations. Of the negative trials over 80% were comprised of responses where the infant either kept his eyes on the experimenter or looked down at the highchair table top.

The proportion successfully responding may well be depressed by the strangeness of the setting although the incidence

Table 1 Percentage of children judged as following line of regard in one or both trials

Age (months)	No. infants	% Showing positive response
2–4	10	30
5–7	13	38.5
8–10	6	66.5
11–14	5	100

of following line of regard is enhanced by pointing and exclamatory vocalisations such as "Oh look". By 11 months, however, some children gave unequivocal positive responses to eye movement alone. Some (from 6 months) even followed line of regard when the experimenter and mother, not interacting with the infant, conversed a metre away from the child.

It is possible that the ability to orient with respect to another has implications for Piaget's¹ more complex notions of the egocentric child. In so far as mutual orientation implies a degree of knowledge in some form about another person's perspective then the child in its first year may be considered as less than completely egocentric. The source of such abilities (for example, imitation) remains to be investigated but utilisation of another's gaze direction may be a very basic process. Cooke's Hartebeeste (*Alcelaphus buselaphus*) on hearing a danger signal raise their heads, look at the signaller and orient in the direction he is facing (M. Stanley-Price, personal communication). Such observations need careful investigation but it may not be entirely unexpected that human infants should also have greater abilities than has been supposed (for example, Schaffer²).

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Role of juvenile hormone esterases and carrier proteins in insect development

THE discovery of a binding protein, specific for juvenile hormone (JH)¹ in the haemolymph of the tobacco hornworm, *Manduca sexta*, suggested that this protein carries JH molecules from the secretory organs (corpora allata) to the target sites. We report here that the binding protein provides the hormone total protection from degradative enzymes present in the haemolymph throughout early larval life^{2,3}. Just before the start of pupal differentiation, however, a new esterase appears in the haemolymph which is able to hydrolyse the protein-bound hormone. We have also found that the binding protein at concentrations of 10^{-8} M strongly enhances the development-inhibiting action of JH on wing disk tissue *in vitro*. These properties, taken together, demonstrate a carrier role for the binding protein, and give new insights into the control of hormone levels in the haemolymph.

Two closely related forms of the binding protein (CP-1 and CP-2) were purified to homogeneity by gel filtration on Sephadex G-100 (0.005 M Tris-HCl, pH 7.3, 0.1 M NaCl), followed by ion-exchange chromatography on Biogel DEAE (0.005 M Tris-HCl, pH 8.3, 0.1–0.14 M NaCl gradient) and preparative isoelectric focusing (pH 4–6, 1% Ampholine, 500 V, 72 h). The binding proteins elute as a single peak from the Sephadex column but are separated on the ion-exchange column and are finally purified by isoelectric focusing. Under these experimental conditions, CP-1 ($pI = 4.8$) was the major binding protein (> 75% of the total hormone binding capacity) and was used for subsequent studies. The hormone-protein complex formed with pure CP-1 and that formed in crude haemolymph showed that same dissociation constant ($K_D \approx 10^{-7}$ M)¹ and slow dissociation rate ($t_{1/2} = 15$ min).

The JH hydrolytic activity in the haemolymph of fifth instar larvae can be separated into two distinct peaks by gel filtration on Sephadex G-100 (0.005 M Tris-HCl, pH 7.3, 0.1 M NaCl). The activity in peak I (molecular weight $\approx 10^6$) is totally inhibited by 10^{-4} M diisopropylphosphorofluoridate

Table 1 Carrier protein protection of JH against hydrolysis by esterase fractions isolated from haemolymph of fifth instar *M. sexta* larvae

Esterase fraction	Hydrolysis of JH (%)		Protection (%)
	–CP*	+CP†	
Peak I	43	0	100
JH-esterase A	74	70	5

Pure synthetic *Hyalophora cecropia* JH' (methyl *trans,trans,cis* 3, 11-dimethyl-7-ethyl-10,11-epoxytrideca-2,6-dienoate) labelled in the chain (7-ethyl-1,2-³H, 67 mCi mg⁻¹ (New England Nuclear Corp.) was used. Reaction products were separated and identified by thin-layer chromatography according to the method of Slade and Zibitt⁴ and quantitated by liquid scintillation counting.

*Incubation for 15 min at room temperature of 20 μ l esterase and 10 μ l JH (8×10^{-7} M) followed by addition of 10 μ l carrier protein (4×10^{-6} M), incubation for 2 min and then addition of 10 μ l charcoal (4.5 μ g) to remove unbound JH. Charcoal removed by centrifugation at 8,000g for 2 min. Supernatant applied immediately to thin-layer chromatographic plate for analyses.

†Same procedure as described for –CP but CP and JH were pre-incubated for 2 min before addition of esterase.

(DFP) (pH 7.0, 15 min) whereas that in peak II (molecular weight $\approx 6 \times 10^4$) is unaffected under the same conditions. Using this concentration of DFP, the crude haemolymph can be assayed for the two types of esterases. Enzyme class II has been further purified by preparative isoelectric focusing (pH 4–6, 1% Ampholine, 500 V, 72 h) and three closely related forms (JH-esterases A, B and C) were separated. All three forms show equal rates of hydrolysis of JH, and JH-esterase A was routinely used for our experiments.

The hydrolysis of the hormone by JH-esterase A was measured in the presence and absence of binding protein CP-1. Table 1 shows that none of the enzymes of family I can hydrolyse JH complexed to CP-1. On the other hand, JH-esterase A hydrolyses the hormone even in the presence of a large excess of CP-1. In an attempt to correlate the presence of JH-esterases with the levels of JH in the insect, we examined the haemolymph from fourth instar larvae and found that esterases of type II are virtually absent. In view of these results it was of interest to study the level of type II esterases during the critical premetamorphic period of the fifth instar. Figure 1 shows that the level of the JH-esterases increases considerably and reaches a peak on day 5 of the fifth instar while type I esterases show relatively little variation during the same period.

These results suggest important functions of both the binding protein and JH-esterases in the regulation of the JH level in the haemolymph during larval development. In the early instars, where high levels of JH are required, the binding protein is necessary to protect JH from degradation by type I esterases. Because of the continuous presence of these esterases, only JH complexed to the binding protein can reach the target tissues and thus the binding protein is the true carrier in the haemolymph. In the fifth instar, on the other hand, type II esterases could constitute the most important factor in lowering the JH level in the haemolymph, thus permitting metamorphosis.

To test further the participation of the carrier protein in the inhibitory action of JH in insect development, we used the technique of Oberlander and Tomblin⁴. This method measures the action of JH in terms of the inhibition of ecdysone-induced cuticle deposition in tissue cultures of imaginal wing disks from the Indian meal moth, *Plodia interpunctella*. Table 2 shows the considerable synergistic action exerted by low levels ($< 10^{-8}$ M) of *M. sexta* carrier protein on JH inhibition of *Plodia* wing disk metamorphosis. The protein alone has no effect in this system. Thus the carrier protein acts not only in haemolymph during transport of the hormone but also at the target tissue. Because of the high level of hormone and the very low level of carrier protein (well below K_D) required to evoke a large synergistic effect, it is unlikely that this is simply a protective effect of the carrier against degradative enzyme, which is known to occur in disk tissue⁵. Rather, the

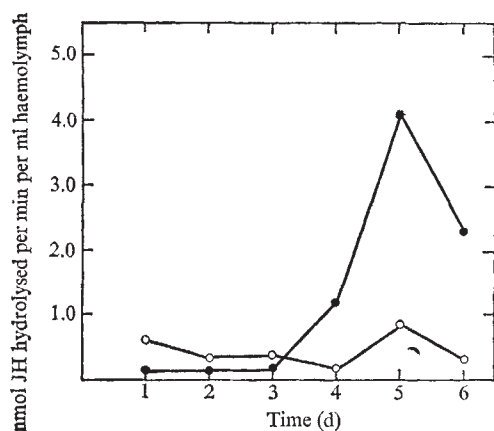


Fig. 1 Activity of general and JH-esterases in the haemolymph of fifth instar *M. sexta* larvae of different ages. Haemolymph was collected as previously described¹ and kept at -20°C until used. Activities were assayed using a newly developed charcoal assay method and specially prepared *M. sexta* JH (methyl *trans, trans, cis* 3,7,11-trimethyl-10,11-epoxydodeca-2,6-dienoate) labelled in the ester methyl group (methyl- ^3H , 8.3 mCi mmol $^{-1}$), (L.L.S., K.J.K., F.J.K., and J.H.L., unpublished). The reaction mixture was composed of 50 μl of JH solution ($4.45 \times 10^{-5}\text{ M}$) in 0.1 M phosphate buffer, pH 7.0, 10 μl of 10% methanol and 50 μl of haemolymph in a 5 ml centrifuge tube. The reaction was quenched with 10 μl glacial acetic acid after which 100 μl charcoal suspension (0.045 mg) was added. The sample was mixed thoroughly and allowed to stand for 20 min and then centrifuged at 15,000g to sediment the charcoal. An aliquot of the supernatant was removed and the amount of radioactive methanol liberated was determined by liquid scintillation counting. DFP-inhibited samples (●) represent JH-esterase activity while non-DFP-inhibited samples represent total JH hydrolytic activity in the haemolymph. General esterase activity toward JH (○) is the difference between JH-esterase and total JH hydrolytic activities. Gut purging preparative to metamorphosis occurs on day 7 in our *M. sexta* colony.

carrier probably serves a function in hormone recognition or transport at the target tissue. High molecular weight lipoproteins have been suggested as JH carriers in saturniid moth pupae and adults⁶ and in adult locusts⁷. In the *Plodia* tissue culture system, preliminary results indicate that the lipoproteins from *Hyalophora cecropia* and *P. interpunctella* had no observable synergistic effect.

By their ability to degrade carrier-bound hormone and by the schedule of their appearance in the haemolymph, the JH-esterases of type II can lower the JH level at the critical time for the induction of metamorphosis. Because non-hydrolysable JH analogues are considerably more active than JH itself in bioassays⁸, it is possible that the JH-esterases are the determining factor in the control of metamorphosis.

Table 2 Effect of *M. sexta* carrier protein on cuticle deposition in cultured imaginal wing disks of *Plodia interpunctella*

[JH]* $\times 10^4$ M	Inhibition of cuticle deposition (%)			
	CP absent	4×10^{-9} M CP	8×10^{-9} M CP	
0	0	0	0	
1.7	15†	—	—	
2.1	28†	50†	100†	
4.2	30	70	—	

In each experiment the mesothoracic wing disks were removed from 10 *Plodia* larvae weighing 12–15 mg. Each pair of disks was divided between experimental and control culture dishes. Fat body was removed from the same larvae and was present in all culture dishes. Either 200 or 400 μl of modified Grace's medium was used in glass micro-dishes containing 10^{-6} M β -ecdysone. Unless otherwise noted, 10 wing disks were used for each experiment. Imaginal disks in this culture system produced tanned cuticle which was scored using a dissecting microscope.

*The juvenile hormone was a mixture of geometric isomers of *Cecropia* juvenile hormone provided by Hoffman-LaRoche.

†Twenty disks were examined.

‡Forty disks were examined.

It is tempting to extend our conclusions to insects other than *M. sexta* since a similar low molecular weight carrier protein has been detected in larvae of *P. interpunctella*⁹. In addition, we have tentatively identified a low molecular weight carrier in *H. cecropia* larvae. The timetable for metamorphosis in all holometabolous insects is very similar and thus a degradative enzyme specific for the hormone-carrier complex could indeed be universally involved in the control of metamorphosis.

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A neuroendocrine feedback mechanism in the insect moulting cycle

THE principles of neuroendocrine integration and regulation developed by the Scharrer^{1,2} are central to current concepts of the regulation of the hypothalamo-hypophyseal system and are applicable to all neurosecretory systems. The importance of these principles in the regulation of the analogous system in insects (the protocephalic corpus cardiacum-allatum system) has, however, been little studied. Here I illustrate certain general functional parallels in the regulation of the vertebrate and insect systems by reference to work on the regulation of the neurosecretory pathway controlling moulting in *Rhodnius prolixus*.

Classical studies^{3,4} have shown that moulting in fifth instar *Rhodnius* is initiated at feeding by the release of a neurosecretory 'brain hormone' which is necessary for 6–8 d after feeding if the moulting process is to proceed to ecdysis. After this 'critical period' the continued release of 'brain hormone' is unnecessary and the secretory behaviour of the medial neurosecretory cells (MNC), which produce it, changes at about this time⁵. The principles of neuroendocrine integration suggest that this change would be implemented after appropriate information had been received by the brain indicating the effectiveness of its released neurosecretory material (NSM). As the principal known effect of this NSM is stimulation of the production of the moulting hormone, ecdysone, experiments have been devised to determine whether the changes in the cytological behaviour on the MNC about the time of the 'critical period' are mediated by the increased haemolymph titre of ecdysone. If this is the case, it should be possible to induce

them prematurely by providing the insect with a supply of ecdysone before its endogenous course of the hormone has become fully active.

In fifth instar *Rhodnius* decapitated 8 d after feeding all known endocrine centres are removed except those producing ecdysone, and the release of ecdysone continues⁶. *Rhodnius* decapitated at 8 days therefore provided insects with intact cerebral neuroendocrine systems early in their moulting process (1 d after feeding) to a sustained prematurely high physiological titre of ecdysone, using parabiosis. When serial sections of the brain (stained with paraldehyde fuchsin) from insects after 7 d of parabiosis (that is, on the eighth day after feeding) were examined, the MNC had been induced to switch over from their normal sequence of cytological changes to those characteristic of the older insect⁷. In particular, a massive number of Type 2a cells is produced during a period when they are otherwise absent, and there is a corresponding decrease in the number of cell Types 2 and 1 (Table 1). Such changes are induced by the haemolymph of donor insects which have passed the 'critical period' but not by that of insects which have yet to reach it. Thus the changes observed in the MNC during the normal moulting cycle⁸ are not an immutable sequence of events but respond dramatically to changes in the composition of the circulating haemolymph.

More direct evidence that ecdysone is the factor responsible for these effects on the MNC has been sought using synthetic hormones. The brain was exposed to a prematurely elevated titre of synthetic ecdysone by injection of 1 μ g β -ecdysone in 1 μ l sterile Ringer solution at 1 d and 3 d after feeding. The two separate injections were given in an attempt to maintain high ecdysone titres from 1–8 d after feeding and minimise any inactivation of the hormone by the fat body⁹. The MNC were examined on the eighth day as before. Ringer-injected controls were normal but the MNC of insects receiving β -ecdysone were strikingly similar to those of insects exposed to the hormone using parabiosis (Table 1).

Ecdysone administration clearly influences the cytology of the MNC. The observed effects of ecdysone are not normally encountered before the 'critical period' but are characteristic of normal insects which have passed it³ and which contain the hormone^{6,9}. Therefore, the changes in the cytological behaviour of the MNC which occur at the 'critical period' in normal insects are probably brought about by ecdysone. This indicates a feedback relationship between the prothoracotrophic 'brain hormone' and ecdysone: the behaviour of the MNC producing the 'brain hormone' changes with the increased titre of ecdysone, the production of which it stimulates. These changes are normally accomplished at the 'critical period', after which the moulting process is independent of the brain: but if a premature independence from the 'brain hormone' is experimentally impressed on the insect by an exogenous supply of ecdysone, the MNC behave as though the period during which such independence is normally achieved, had arrived.

Reasoning has been presented elsewhere that the cytological changes induced by ecdysone indicate that the hormone causes an inhibition of release of NSM from the MNC

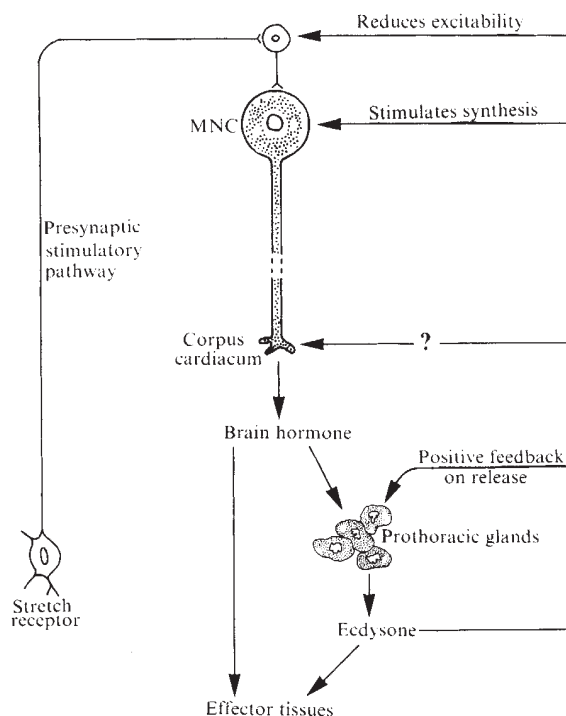


Fig. 1 Schematic diagram of the proposed interactions between the cerebral neurosecretory system and ecdysone during moulting in *Rhodnius*.

coupled with stimulation of synthesis of new material⁷. Hence the effect of ecdysone on the MNC is apparently a negative feedback on release of NSM coupled with a stimulation of synthesis. Such an arrangement possesses striking functional similarities with the so-called 'short-loop' feedback mechanisms familiar (though controversial) in the regulation of the anterior pituitary whereby adenohypophyseal hormones influence production of their respective releasing factors in hypothalamic nuclei^{10,11}. The adenohypophysis and prothoracic gland are comparable as endocrine effectors of neurohormones¹² and it now seems that both glands provide the neurosecretory cells which regulate them with humoral feedback information.

The following scheme suggests how this feedback mechanism may operate in the normal moulting cycle of *Rhodnius* (Fig. 1). Abdominal distension following feeding excites stretch receptors which discharge to the brain^{4,13}, providing presynaptic stimulation of the MNC and causing release of NSM⁸. The principal effect of the NSM is to stimulate production of ecdysone which promotes moulting in the tissues. Ecdysone seems to have a positive feedback on its own production^{14,15}, thereby amplifying the hormonal instructions from the brain. By the time ecdysone production peaks, release of 'brain hormone' is greatly reduced⁴ and the head is no longer necessary for moulting⁴. This decline in 'brain hormone' release is not due merely to a decline in the afferent stimuli favouring release, however,

Table 1 Effects on the MNC of premature exposure to ecdysone by parabiosis and by injection of synthetic hormone

Cell type	Type 1	Type 2	Type 2a	Type 3	Type 4	Total
Normal insect at day 8	4.2 $\pm$ 0.2	6.4 $\pm$ 0.2	0.2 $\pm$ 0.1	3.2 $\pm$ 0.3	5.0 $\pm$ 0	19.1 $\pm$ 0.3
Parabiosis (from 1 to 8 d) to a decapitated insect producing ecdysone	1.8 $\pm$ 0.2	2.6 $\pm$ 0.3	5.8 $\pm$ 0.5	3.2 $\pm$ 0.4	5.3 $\pm$ 0.1	18.7 $\pm$ 0.4
Ecdysterone (1 μ g) injected at days 1 and 3	2.1 $\pm$ 0.5	4.1 $\pm$ 0.6	6.4 $\pm$ 0.5	2.6 $\pm$ 0.3	5.0 $\pm$ 0.1	20.3 $\pm$ 0.6

Figures are the mean number ($\pm$ s.e.) of the five cytologically distinct cell types present in the MNC on day 8 after feeding, following the treatments described

because the time course of the process is influenced by ecdysone, the increasing titre of which seems to act back on the brain where release is inhibited and synthesis of NSM promoted. Indeed, an engorged *Rhodnius* remains noticeably distended for several weeks after feeding and the abdominal stretch receptors show a maintained adapted discharge rate¹⁸. It follows that at the time when the effects of ecdysone on the MNC normally become apparent (at 6–8 d), neural stimuli favouring release would still be arriving at the brain and the neurosecretory system would have to 'decide' between these conflicting neural and humoral instructions. The significance of the MNC as the 'final common path'¹⁷ of this process of neuroendocrine integration is most obvious in the experiments where ecdysone is provided 1 d after feeding; the rate of release of NSM in response to the neural stimuli is normally very high at this time³ but these stimuli cease to be effective when ecdysone is provided. This suggests that the feedback effect of ecdysone may occur either directly on the MNC or on neurones concerned with their presynaptic control, to reduce their sensitivity to presynaptic stimulation (Fig. 1). Indeed, there is some evidence that ecdysone directly depresses the firing rate of insect central neurones¹⁸. Similarly, the effect of adenohypophyseal hormones on the hypothalamus may be on the cells elaborating releasing factors or on neurones controlling them^{10,11}. Steroid sensitivity is apparently not a general property of central neurones, however, as not all hypothalamic neurones show it¹⁹ and in *Rhodnius* some of the cell types in the MNC respond to ecdysone while others seem to be unaffected (Table 1).

The reported inhibition of release of NSM at the 'critical period' is thus explicable in terms of selective modulation of central excitability by ecdysone; the simultaneous promotion of synthesis by ecdysone must involve other mechanisms which are provisionally assumed to include direct action of ecdysone on the MNC (Fig. 1). Although tentative because of the indirect nature of the evidence, the above scheme illustrates the mutual interdependence of insect hormones and the importance of neuroendocrine integration in their regulation.

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Role of growth hormone in glycosaminoglycan synthesis by articular cartilage

LITTLE is known of the factors which regulate the turnover of the glycosaminoglycans, chondroitin sulphate and keratan sulphate,

in articular cartilage. Studies using costal cartilage¹ suggest that growth hormone (somatotrophin) has little direct action on glycosaminoglycan synthesis. Instead its action is thought to be mediated by the liver² which produces a different hormone, somatomedin, previously known as 'sulphation factor' because it stimulates the incorporation of ³⁵SO₄ into costal cartilage.

These conclusions are not necessarily true for articular cartilage. Denko and Bergenstal³ found differences between the incorporation of ³⁵SO₄ by costal cartilage and the cartilage of the tibial 'cap' in young rats. For example, following hypophysectomy, incorporation of ³⁵SO₄ by costal cartilage was depressed, but no change was observed for the tibial 'cap'.

That such differences should exist is not surprising, for although costal and articular cartilage resemble each other in structure, their function is entirely different. Furthermore, it is necessary to distinguish between articular cartilage and epiphyseal cartilage and the tibial 'cap' is unsatisfactory because it includes both.

We carried out two separate experiments to investigate the action of growth hormone on articular cartilage. *In vitro* studies enabled us to investigate the action of bovine growth hormone on bovine articular cartilage. Of necessity we had to use smaller animals for *in vivo* studies and we selected the rat, as bovine growth hormone is known to be biologically active for this species.

In the *in vivo* studies bovine growth hormone (NIH-GH.B17) was given by subcutaneous injection for 14 d to mature male rats. During this treatment these 'plateaued' rats showed a gain in weight, compared with a control group which showed a slight fall. After 14 d, each animal was given 100 µCi ³⁵S-labelled Na₂³⁵SO₄ by intraperitoneal injection and killed 24 h later. The distal femora and proximal tibiae from each animal were decalcified and sections through their joint surfaces examined by the method of autoradiography. There was no detectable difference between the group treated with growth hormone and the controls with regard to labelling of the articular cartilage. Slivers of articular cartilage were cut from the proximal humerus of the same two groups of animals for quantitative study. These samples were weighed by an electrobalance and dissolved by boiling with 23 M formic acid; radioactivity was determined using a Packard Tri-Carb liquid scintillation counter. Counts for each sample were divided by their weight to give specific activity. This showed no significant difference between the two groups.

In such an *in vivo* study, it is not possible to distinguish between the effects of growth hormone itself and somatomedin which is presumably released by the liver in response to growth hormone injections. These factors were investigated separately *in vitro*, using a medium containing somatomedin prepared by previous incubation of growth hormone with liver slices⁴. Optimal conditions for glycosaminoglycan synthesis were obtained by organ culture of bovine articular cartilage in 20% oxygen and using a chemically defined medium⁵. The articular cartilage was obtained from 6-month-old calves, being from the same species as the growth hormone (NIH-GH.B17).

Organ culture of articular cartilage was performed simultaneously using eight culture chambers, divided into four groups, differing only in the nature of the culture medium⁴. Group A, Eagle's MEM; group B, Eagle's MEM containing bovine growth hormone (concentration 1.0 µg ml⁻¹); group C, Eagle's MEM incubated beforehand with liver slices; group D, Eagle's MEM plus bovine growth hormone (concentration 1.0 µg ml⁻¹) incubated beforehand with liver slices.

Four samples of cartilage, each about 1 mg dry weight, were removed from each chamber after 24, 48 and 72 h. This amount of material is adequate for fractionation of glycosaminoglycans on the microscale⁶.

Two separate experiments were performed, using different donor animals to provide the articular cartilage. Samples were pooled from each of the groups, A, B, C and D, so that the final values represent analysis of 16 separate pieces of articular cartilage for each group.

Table 1 Specific activities of fractions from CPC cellulose microcolumns

Eluting solution	24 h				48 h				72 h			
	A	B	C	D	A	B	C	D	A	B	C	D
0.3 M NaCl	1,256	2,077	959	748	1,314	636	407	828	1,374	1,297	664	581
0.25 M MgCl ₂	4,666	4,197	1,289	1,568	4,111	1,381	788	756	2,668	2,089	930	1,477
0.3 M MgCl ₂	1,898	1,098	1,008	1,201	1,918	492	493	523	1,550	563	598	877
0.35 M MgCl ₂	979	667	519	542	872	313	189	321	788	334	469	371
0.4 M MgCl ₂	882	479	415	505	588	134	150	201	454	276	237	260
0.45 M MgCl ₂	869	495	309	474	433	111	114	115	492	198	208	189
0.5 M MgCl ₂	1,377	1,075	472	472	427	120	134	100	506	196	325	263
0.6 M MgCl ₂	4,305	2,213	1,794	2,215	778	165	262	100	499	228	574	676
2.0 M MgCl ₂	1,585	2,102	2,430	2,144	364	125	556	983	259	075	510	948
6 M HCl	375	238	342	263	106	283	126	046	228	0	279	318
Total chondroitin sulphate	1,290	828	533	630	689	245	185	131	716	315	337	332
Highly sulphated keratan sulphate	954	828	1,233	895	273	170	354	522	240	330	388	672

The values shown are means of two calculations of specific activity (c. p. m. divided by hexosamine content in μg) for each fraction eluted from the CPC cellulose microcolumns. The different experimental groups, A, B, C and D are explained in the text. Chondroitin sulphate of increasing chain length is eluted by the 0.25 M to 0.6 M MgCl₂ solutions and highly sulphated keratan sulphate by the 2.0 M MgCl₂ and 6.0 M HCl fractions. The mean specific activity for these two glycosaminoglycans was determined by dividing the sum of counts for each of their fractions by the sum of the hexosamine content of these fractions.

After their removal from the culture chambers, the cartilage samples were incubated in Tyrode's solution, containing 0.5 μCi $^{35}\text{SO}_4$ ml⁻¹. After 1 h they were removed and further enzyme activity prevented by immersion in monoiodoacetic acid. The samples were washed to remove unbound $^{35}\text{SO}_4$, dehydrated in acetone and dried in air to constant weight. Following digestion by papain, the glycosaminoglycans were fractionated on cetyl pyridinium chloride (CPC) cellulose microcolumns⁶, using eluting solutions as shown in Table 1. All analyses were performed in duplicate. Chondroitin sulphate is eluted by the magnesium chloride solutions 0.25 M to 0.6 M and highly sulphated keratan sulphate by 2 M magnesium chloride and 6 M HCl. The first fraction obtained by elution with CPC contains keratan sulphate with glycopeptides and some unbound sulphate. These components were separated using ecteola cellulose microcolumns⁷, keratan sulphate being eluted by 0.4–1.2 M NaCl solutions. Characterisation of these fractions from calf articular cartilage has been previously reported using electrophoresis on cellulose acetate membranes⁸. Each fraction

was divided into aliquots for determination of hexosamine content and scintillation counting.

The results shown in Tables 1 and 2 are indices of the synthesis rate of each fraction, that is, c.p.m. divided by the weight of hexosamine in micrograms ('specific activity').

The addition of growth hormone to the culture medium (group B) resulted in diminished incorporation of $^{35}\text{SO}_4$ for all fractions after each of the time intervals studied. This applies equally to all fractions of chondroitin sulphate (0.25 M to 0.6 M MgCl₂ fractions) and keratan sulphate (0.4 M to 1.2 M NaCl fractions plus the 6 M HCl fractions). A similar depression of $^{35}\text{SO}_4$ is seen for group D (previous incubation of medium with growth hormone plus liver slices), but with the exception of highly sulphated keratan sulphate (2.0 M MgCl₂ and 6 M HCl fractions from the CPC columns). After 48 and 72 h, this highly sulphated keratan sulphate showed an enhanced incorporation of $^{35}\text{SO}_4$, presumably in response to somatomedin, as no such change was seen for group B or group C (previous incubation with liver slices only).

The design of this experiment was very similar to that of

Table 2 Specific activities of fractions from ecteola cellulose microcolumns

Eluting solution	24 h				48 h				72 h			
	A	B	C	D	A	B	C	D	A	B	C	D
H ₂ O	0	0	0	0	0	0	0	0	171	900	0	173
0.02 M HCl	0	0	0	0	066	0	0	0	1,928	354	0	040
0.3 M NaCl	829	477	303	224	1,106	398	086	188	2,538	1,589	347	484
0.4 M NaCl	1,339	813	422	236	2,193	586	353	375	2,519	1,047	384	413
0.5 M NaCl	729	717	271	057	1,450	329	176	240	1,251	466	228	294
0.6 M NaCl	362	217	0	0	142	167	074	099	443	148	053	115
0.7 M NaCl	141	030	0	0	179	0	0	0	204	0	072	034
0.8 M NaCl	117	211	0	0	121	0	0	0	060	0	0	074
1.2 M NaCl	0	0	0	0	0	0	0	0	0	0	0	0
6 M HCl	0	0	0	0	0	0	0	0	0	0	0	0
Total keratan sulphate	493	298	124	062	708	157	092	109	1,210	402	169	197

The values shown are means of two calculations of specific activity for each fraction eluted from the ecteola cellulose microcolumns on refractionation of the CPC fractions obtained from the CPC cellulose microcolumns. Keratan sulphate is eluted by the 0.4 M to 1.2 M NaCl fractions and a mean specific activity was determined by dividing the sum of the counts for these fractions by the sum of their hexosamine content. Unbound $^{35}\text{SO}_4$ was removed from the ecteola cellulose columns by previous incubation with 0.1 M NaCl, repeated six times to give six fractions which had no significant hexosamine content. The zero for some of the fractions shown in this table represents a count below that of a standard blank salt solutions of the same molarity.

McConaghey⁴, who found that incorporation of ³⁵SO₄ by costal cartilage was slightly increased by the same purified growth hormone and markedly increased by somatomedin, prepared using liver slices. It is clear that articular cartilage behaves quite differently in its response to growth hormone, which, *in vitro*, depresses glycosaminoglycan synthesis. The response of articular cartilage to somatomedin was similar to that of costal cartilage only for highly sulphated keratan sulphate. It is very likely, however, that the medium used in group D still contains growth hormone not metabolised by the liver slices. This residual growth hormone could have obscured the action of somatomedin on the synthesis of chondroitin sulphate and keratan sulphate.

Whatever the effect of somatomedin, there can be no doubt that growth hormone itself has a direct action on articular cartilage *in vitro*. That action is to decrease glycosaminoglycan synthesis.

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Purification of human uterine progesterone receptor

It is now generally accepted that before the physiological response to the androgens¹, oestrogens², glucocorticoids³ and progesterone⁴⁻⁷ can be manifested, these hormones must complex with specific receptors in their target tissue. Since progesterone is important both in the mechanisms associated with transport of the ovum through the oviduct^{8,9} and in preparing the endometrium for implantation of the blastocyst¹⁰, a greater understanding of the action of the hormone-receptor complex at the molecular level will lead to a more detailed knowledge of the mechanisms involved in reproductive biology. This understanding may be utilised to develop new contraceptive methods and to investigate the genesis and treatment of endometrial adenocarcinoma.

To study the molecular events associated with the function of progesterone and to determine the topography of the receptor site, it is necessary to obtain the receptor-hormone complex in a pure form. We report here the isolation and purification of the progesterone receptor from human uterus. A similar receptor has also been studied from the human oviduct (C.A.I., unpublished), and translocation and binding to oviduct nuclei has been demonstrated. Purification was achieved using a combination of ammonium sulphate fractionation, affinity chromatography and ion-exchange chromatography. The overall purification from the 105,000g supernatant fraction from a

crude tissue homogenate is approximately 8,000-fold. Since the cytosol fraction is an approximately fivefold purification over the whole cell we estimate a 40,000-fold purification for the progesterone receptor with respect to the intact cell. The concentration of progesterone binding sites in the crude cytosol is 3×10^{-9} M per mg protein. The pure receptor sediments at 3.7S on sucrose gradient centrifugation and migrates as a single band of molecular weight 110,000 on SDS polyacrylamide gel electrophoresis. It has not yet been determined, however, whether more than one subunit of the same molecular weight is present. These data will be reported in detail elsewhere.

The specificity of the ammonium sulphate-purified receptor (Table 1) showed the following relative affinities: progesterone > 11-desoxycorticosterone > 5 α -dihydroprogesterone > 5 β -

Table 1 Comparisons of hormone binding to the progesterone receptors from human, chick and hamster target tissues

	Human	Chick ^a	Hamster ^a
Progesterone	100	100	100
5 α -Dihydroprogesterone	12	86	20
5 β -Dihydroprogesterone	6	15	4
11-Desoxycorticosterone	28	94	64
Substance S	7	7	11
Corticosterone	5	3	3
Cortisol	<1	<1	<1
Testosterone	2	5	4
Oestradiol	<1	<1	1

Tissues were segmented and rinsed in ice-cold saline solution, weighed and homogenised in four volumes (w/v) TESH buffer, 0.01 M Tris-HCl, 1.0 mM Na₂EDTA and 0.012 M thioglycerol (pH 7.4). The homogenate was centrifuged at 27,000g for 10 min and the supernatant was recentrifuged at 105,000g for 1 h to obtain the cytosol fraction. Glycerol was added to the buffer to a concentration of 40% when human uterine tissue was used. For the competition assay a series of tubes were prepared containing cytosol, ³H-progesterone and unlabelled steroid competitors in TESH buffer. The assay tubes were incubated at 0°C for 16-20 h and ³H-progesterone binding was measured by charcoal adsorption. Each assay point was determined twice. A standard curve for the competition of unlabelled progesterone was included in each assay using five progesterone concentrations usually in the range 3.5×10^{-9} to 7.1×10^{-7} M. Four or five concentrations of each competitor were tested using a range between 3×10^{-9} and 7×10^{-7} M. The competitor concentrations were chosen to provide a linear portion on a semi-log plot which would cross the point of 50% competition. From this plot, the concentrations of unlabelled progesterone and of steroid competitors which reduced ³H-progesterone binding by 50% were determined. These quantities were converted to molar concentrations and the effectiveness of the competitor was established using the ratio: unlabelled progesterone concentration for 50% competition/competitor concentration for 50% competition. This ratio was multiplied by 100.

dihydroprogesterone > corticosterone > testosterone >> oestradiol > cortisol. The relative affinity of cortisol shows that the most probable contaminant, cortisol binding globulin, is absent. A comparison of these specificities with those obtained from crude cytosol receptors of chick oviduct¹¹ and hamster uterus¹² (Table 1) shows that the partially purified human uterine receptor is the more selective. It was not possible to determine specificity by competitive assay for progesterone binding sites at subsequent steps in the purification since the receptor is saturated with labelled progesterone during its elution from the affinity column. Because of the lability of the receptor, it has not yet been possible to remove the hormone and retain the native activity of the receptor. Scatchard analysis shows, however, that only molecules containing binding sites specific for progesterone are retained by the affinity resin.

The affinity resin was prepared by coupling denatured bovine serum albumin to cyanogen bromide-activated Sepharose¹³ followed by reaction with 11-desoxycorticosterone hemisuccinate in the presence of 1-ethyl-3-(3-dimethylaminopropyl)

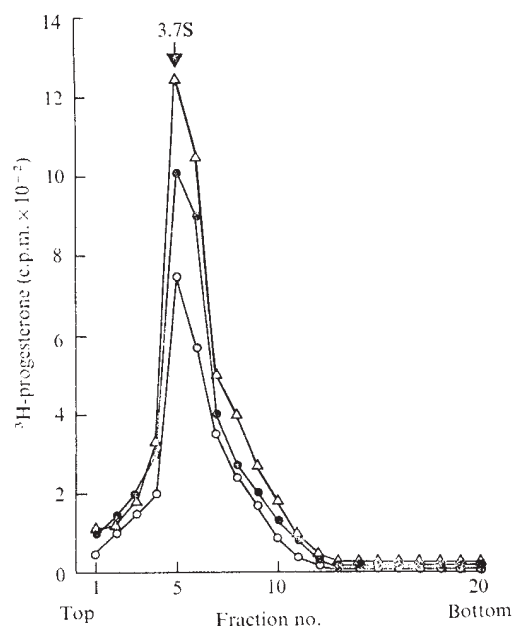


Fig. 1 Sucrose gradient ultracentrifugation analyses of ^3H -progesterone-treated: Δ , human uterine cytosol; $\bullet$, receptor obtained after affinity chromatography; $\circ$, nuclear extract (0.4 M KCl) from human oviduct. Samples (0.2 ml) were layered on linear 5–20% (w/v) sucrose gradients made up in 10% glycerol, containing 0.05 M Tris, 1 mM EDTA and 0.01 M thioglycerol at pH 7.5, and then centrifuged for 17 h at 216,000g. ^{14}C -ovalbumin (3.7S) was used as a sedimentation standard.

carbodiimide hydrochloride. After thoroughly washing each millilitre of resin with 200 ml dioxane followed by 21 80% methanol/water, the capacity of the resin is 0.21 nmol per ml packed resin and its dissociation constant for the receptor is 10^{-9} M (the dissociation constant of progesterone for its receptor is $\sim 10^{-9}$ M). Cortisol (10^{-6} M) was added to the ammonium sulphate-precipitated receptor prior to incubation with the affinity resin, so that binding of the resin to corticoid receptors and possibly certain steroid metabolising enzymes would effectively be negated. After incubation for 12 h at 2°C each millilitre of resin was washed with 200 ml of buffer containing a high salt concentration (0.4 M KCl) to remove relatively loosely associated proteins. Elution of the receptor in batches was accomplished by incubating at 20°C for 30 min in the presence of excess labelled progesterone. The macromolecular bound hormone was isolated by gel filtration and applied to a DEAE-cellulose column and eluted with a salt gradient. The receptor–progesterone complex was eluted in 0.2 M KCl, dialysed, lyophilised and applied to an SDS polyacrylamide gel. Electrophoresis revealed a single band of molecular weight 110,000.

The dissociation constants for progesterone ($K_D \sim 10^{-9}$ M) and the relative affinities of various steroids for the crude and the partially purified receptor are almost identical (Table 1). The crude, the partially purified and the pure receptor all show

similar physical properties: the macromolecule sediments at 3.7S (Fig. 1) and is eluted from phosphocellulose by 0.17 M KCl, from DEAE-cellulose by 0.2 M KCl. A nuclear extract obtained after preincubation of nuclei with labelled receptor also sediments at 3.7S (Fig. 1). Collectively, the data therefore confirm the integrity of the pure progesterone receptor.

Incubation of human oviduct nuclei with receptor– ^3H -progesterone complex, both at 0° and 37°C , showed translocation was occurring. Extraction of the nuclei with a high salt buffer (0.4 M KCl) followed by sucrose gradient centrifugation of the extract demonstrated macromolecular bound hormone sedimenting at 3.7S. It is not surprising that nuclear binding occurs at both temperatures since it has been shown previously in the chick oviduct system that activation of the progesterone–receptor complex by ammonium sulphate alone is sufficient to effect translocation and binding at 0°C (ref. 14). Human oviduct rather than endometrial nuclei were used because of the greater amount of tissue available combined with ease in obtaining pure nuclei.

For the first time, therefore, we have demonstrated the purification of a human uterine progesterone receptor (Table 2), together with its translocation and binding to human target nuclei. Characterisation of the receptor site may enable us to design specific agents to block progesterone binding at this site thus precluding the physiological action of this hormone. In addition, we now have the opportunity to study in detail the mechanism of action of progesterone.

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Table 2 Purification of human uterine progesterone receptor

	c.p.m./mg protein	Yield (%)	Purification (-fold)
Cytosol	2×10^4	—	—
Ammonium sulphate (30%)	8.5×10^4	30	4.3
Affinity column eluent	5.4×10^7	100	2,700
DEAE cellulose–0.2 M KCl	1.65×10^8	10	8,300

Is potassium conductance of cardiac Purkinje fibres controlled by $[Ca^{2+}]_i$?

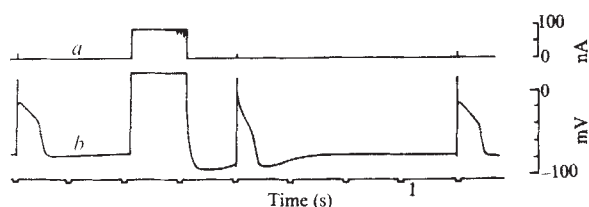
ELEVATED extracellular Ca^{2+} concentration¹, prolonged treatment with high doses of cardiac glycosides², and metabolic inhibition³ shorten the action potential of cardiac muscle. In these conditions the intracellular free Ca^{2+} may be increased and an increase in intracellular Ca^{2+} could produce shortening of the action potential either by affecting the Ca^{2+} inward current or by increasing the potassium permeability⁴. The latter mechanism has been observed in red blood cells⁵ and in the neurones of some species⁶⁻⁸. Here I give the results of studying the effect of iontophoretically injected Ca^{2+} in cardiac Purkinje cells. Such injection led to membrane hyperpolarisation and considerable shortening of the action potential, suggesting that the level of intracellular free Ca^{2+} can affect the potassium permeability in cardiac muscle.

The diffusion coefficient of radiocalcium in skeletal muscle fibres seems to be 100 times less than that in water⁹. This suggests that an effect produced by the electrophoretic injection of Ca^{2+} can only be observed within a small region round the injection site. To ensure that the tip of the microelectrode recording resting and action potentials was placed in the region of elevated $[Ca^{2+}]_i$, in most experiments a single electrode filled with 1 M $CaCl_2$ was used both for injection and for recording¹⁰. A second intracellular electrode (filled with potassium citrate) served to stimulate the preparations at a rate of 15 min⁻¹.

In Fig. 1*b*, the first action potential (AP) illustrates the control values (resting potential (V_m), -76 mV; plateau potential, -16 mV; duration of the action potential (APD), 635 ms). Thereafter an inward directed current pulse (Fig. 1*a*) injected about 0.1 μ Ci from the Ca^{2+} filled electrode; this current depolarised the preparation to +20 mV for 1 s. When the injecting current was switched off the membrane re- and hyperpolarised (V_m -95 mV). One second after injection, the second AP was elicited. The plateau began at a potential more positive (-7 mV) and was shorter than that of the control, the APD being reduced to 380 ms. From the plateau, the membrane repolarised (with almost unchanged speed) to a high negative (-91 mV) diastolic potential. Within the next 2 s V_m declined like an enhanced pacemaker potential and 5 s after injection both V_m and the AP were almost identical with the control.

It could be argued that injecting the current produces not only an increased $[Ca^{2+}]_i$; but also a strong activation of the potassium conductance g_K due to the large depolarisation⁷. To exclude the latter possibility, in five experiments the Ca^{2+} injecting electrode was replaced by a double-barrelled electrode, one side filled with $CaCl_2$, the other with 3 M KCl. The current was injected between the $CaCl_2$ barrel and second intracellular microelectrode filled with potassium citrate without an earth

Fig. 1 Effect of intracellular iontophoretic injection of +0.1 μ Ci (a) from a microelectrode filled with 1 M $CaCl_2$ solution on transmembrane resting and action potential (b). Experiment on sheep Purkinje fibre, superfused by oxygenated Tyrode solution containing (mM) 150 NaCl, 4 KCl, 0.9 $CaCl_2$, 1 Mg Cl_2 , 5 glucose and 10 Tris-maleate buffer, pH 7.4, temperature 37°C. Chart recording, the action potential overshoots are attenuated. The depolarisation during injection was measured by the non injecting K-citrate electrode.



(battery-driven current pump). A third intracellular microelectrode was used for stimulating the preparation. A current of +100 nA out of the $CaCl_2$ capillary, hyperpolarised the membrane from -85 to -92 mV and 0.8 s after the end of injection the APD was shortened from 540 ms (control) to 320 ms. If, after injecting the Ca^{2+} , the membrane was clamped to the control level (-85 mV) for 500 ms, nearly the same reduction of APD (from 540 to 310 ms) was observed.

The resistance of the electrode increased during injection. When more than 0.5 μ Ci were applied the current frequently decreased (R_{ei} more than 800 M Ω , saturation of the current pump⁷). It was then difficult to separate the Ca^{2+} induced hyperpolarisation from an increase in tip potential. The stability of the electrodes was better when they were filled with a Ca^{2+} -EGTA buffer solution (electrodes filled with 0.09

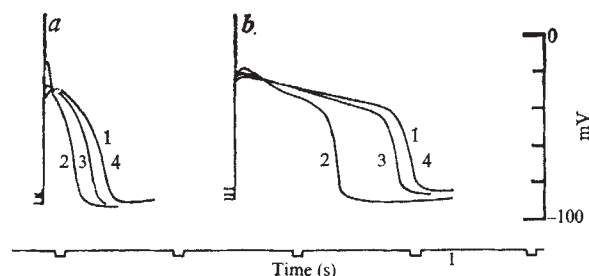


Fig. 2 Transmembrane action potentials. Each panel shows photographically superimposed chart recordings of control action potentials (trace 1 in a, b) and after iontophoresis. a, Injection of +0.3 μ Ci from a microelectrode filled by Ca^{2+} -EGTA buffer solution. Tyrode with $K_0 = 5.4$ mM. Action potential 0.4 s (trace 2), 0.8 s (trace 3) and 2.5 s (trace 4) after the end of the injecting current pulse. b, Injection of +0.3 μ Ci as in a, but at a lower K^+ concentration (4 mM) in the Tyrode solution. Action potential 0.8 s (trace 2) 1.5 s (trace 3) and 3 s (trace 4) after the end of the injecting current pulse.

M $Ca(OH)_2$, 0.1 M EGTA, and 0.1 M Tris; resistance of the electrodes 120-150 M Ω)⁸.

Figure 2*a*, trace 2, shows an AP 0.4 s after injection of +0.3 μ Ci from an electrode filled with Ca^{2+} -EGTA buffer. The injection caused hyperpolarisation and a reduction of the APD from 550 ms (trace 1, control) to 250 ms (trace 2). The configuration of the AP was altered similar to that in Fig. 1. For constant charges of the injected Ca^{2+} , the shortening of the APD was more pronounced the shorter the delay between injection and AP. Thus, 0.8 s after injection, the APD was shortened only by a small amount (Fig. 2*a*, trace 3) and 2.5 s after injection the AP was identical to the control (Fig. 2*a*, traces 1 and 4). This normalisation of the APD could be fitted by a single exponential. From 22 experiments, a time constant of 0.5 ± 0.2 s was calculated for the decay.

In Fig. 2*b* the Tyrode solution contained a K^+ concentration somewhat smaller (4 mM) than in Fig. 2*a* (5.4 mM). Injection of +0.3 μ Ci produced hyperpolarisation of V_m and shortening of APD as in Fig. 2*a*. Both effects however, were more pronounced and lasted longer (time constant for the decay, 1.0 ± 0.2 s; $n, 15$).

When the time interval between injection and AP was constant, the APD was shorter the larger the amount of the injected Ca^{2+} . Currents less than 10 nA did not affect the APD, independent of their duration (presumably the cell can rapidly extrude or sequester such an amount). The formulation of a quantitative relationship was not, however, possible with the present data.

The shortening of the APD on the injection of Ca^{2+} could be explained by a reduction of the Ca^{2+} driving force diminishing

the slow inward current⁷. Viewed in conjunction with the strong hyperpolarisation, however, the most likely explanation is that elevated $[Ca^{2+}]_i$ increased the potassium conductance g_K .

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Role of foetal adrenocorticotrophin during parturition in sheep

It is now well accepted that the lamb foetus plays an important role in the mechanisms which initiate its own delivery. This conclusion is based on several experimental observations. Foetal hypophysectomy or adrenalectomy delays parturition^{1,2}; infusions of adrenocorticotrophin (ACTH) or cortisol, which is the major glucocorticoid in this species, will precipitate premature delivery when infused into the foetus³; normal delivery is preceded by an increase in the concentration of corticosteroids in the foetal blood^{4,5} and an increased production of cortisol by the foetal adrenal⁶.

The simplest hypothesis to explain these changes is that an increase in foetal pituitary secretion of ACTH stimulates the production of cortisol from the foetal adrenal. The cortisol is thought to act on placental enzyme systems to bring about several changes, in particular an increase in maternal plasma oestrogen concentrations^{7,8}, and maternal oestrogens have been shown to rise sharply in the last few hours before delivery⁹. This increase also occurs after the administration of ACTH or glucocorticoids to the foetus⁷.

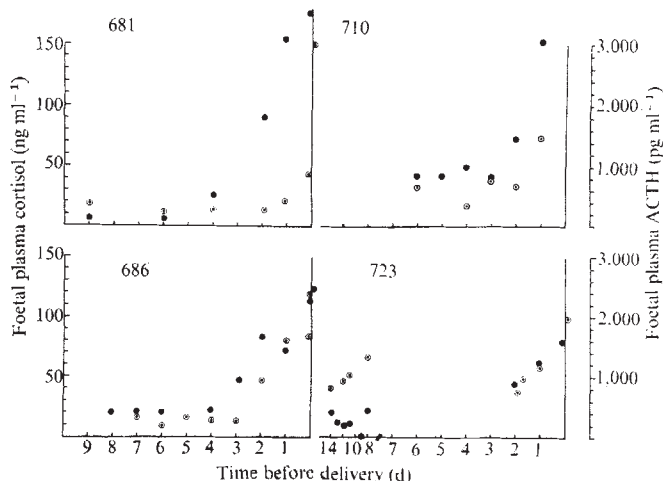
The results obtained from the experiments involving foetal hypophysectomy, however, do not prove that an increased secretion of ACTH by the foetal pituitary is necessarily involved in the initiation of parturition. It is possible that foetal hypophysectomy may delay parturition by lowering foetal plasma ACTH concentrations and thus impairing adrenal maturation and the development of adrenal responsiveness to other stimuli of either foetal or maternal origin. These alternative explanations have been discussed in detail elsewhere⁸. The changes in foetal plasma corticosteroid and ACTH concentrations in the period before parturition have been studied in the foetal sheep using indwelling vascular catheters¹⁰. Foetal plasma ACTH concentrations were reported to be very labile, 100–1,800 pg ml⁻¹, and the authors concluded that when average values were considered there seemed to be a rise in ACTH during the last days before delivery. Sequential values from the same animal for more than three days were available in only two foetal sheep which were delivered vaginally and one of the two ewes was subjected to three bouts of experimental hypoxia. It therefore seemed important to obtain further data from undisturbed fetuses during late gestation and parturition. The experiments reported here were carried out to relate changes in plasma ACTH and cortisol concentrations both to each other and to the timing of delivery.

Seven ewes with singleton pregnancies of 124–130 d gesta-

tional age were used in the present investigation. A foetal venous catheter was introduced into each leg through the lateral tarsal vein. Foetal blood samples were taken at least once daily, if obtainable, throughout the experimental period for the assessment of foetal condition as indicated by blood gas and pH analysis, but no samples for hormone estimations were taken for at least 48 h after surgery. In all samples analysed for ACTH and cortisol concentration, foetal pH was within the normal range (7.32–7.41) except for samples taken during the final stage of labour when the usual fall in foetal pH was observed. Blood gas tensions were normal. Foetal blood was taken into cold syringes, centrifuged immediately and the plasma stored at –20°C. All estimations were performed within 1 month of sampling. Total plasma corticosteroids were measured in most samples by competitive protein binding without chromatographic separation¹¹. Some samples taken from foetuses at different days before delivery were separated on a Sephadex LH20 system¹². When this was done, cortisol accounted for more than 80% of the total corticosteroids as measured by competitive protein binding irrespective of gestational age. This finding is in agreement with the pattern of steroid secretion found when foetal adrenals of different ages are incubated *in vitro*¹³. Corticosteroid concentrations have therefore been designated as cortisol in the present study. Plasma ACTH was measured by radioimmunoassay after extraction on to porous glass¹⁴. Plasma samples of 0.5–1.0 ml were extracted and Third International Standard ACTH (31WS)¹⁵ used as both standard (unlabelled) and iodinated (labelled) hormone.

Two foetuses were each sampled four times over 48 h on days 130–132 of gestation to obtain data on foetal plasma ACTH concentrations before they were used for a separate experiment. Four foetuses were sampled up to the spontaneous delivery of a live lamb (Fig. 1). One further foetus was sampled every 6 h (0000, 0600, 1200, and 1800) for 4 d. A

Fig. 1 Plasma cortisol (●) and ACTH (○) concentrations over the last 14 d of intrauterine life in four chronically catheterised foetal sheep. Although the foetal catheters were patent, it was impossible to obtain blood samples from S723 between 8 and 2 d before delivery. S681 delivered at 147 d gestational age, 11 d after catheterisation; S686 delivered at 133 d gestational age, 9 d after catheterisation; S710 delivered at 139 d gestational age, 11 d after catheterisation; S723 delivered at 151 d gestational age, 23 d after catheterisation.



total of 40 ml of blood was removed in this period and therefore blood loss may have been a contributory factor to the subsequent death of this foetus *in utero*. It was equally possible that death occurred *intra partum* as the increase in foetal plasma cortisol was followed by a rise in maternal plasma oestrogen concentration while the foetus was still alive.

In none of the five foetuses which were sampled sequentially was there any significant increase in foetal plasma ACTH before the preparturient rise in foetal plasma cortisol concentration. In three of the animals, plasma ACTH concentrations were low in the period up to 96 h before delivery (242.5 ± 18.4 pg ml⁻¹; mean $\pm$ s.e.m., $n = 16$). Foetuses 686 and 681 were in this group. Mean plasma ACTH concentration in the other foetuses was 691.8 ± 127.3 pg ml⁻¹ ($n = 9$) in this same period (foetuses 723, 710). For all five foetuses the mean plasma concentration of ACTH for the period up to 4 d prepartum was 435.8 ± 67.6 pg ml⁻¹ ($n = 25$). In the two further foetuses sampled four times between 130 and 132 d, mean plasma ACTH concentrations were 177 and 203 pg ml⁻¹.

In contrast to the absence of an increase in foetal plasma ACTH before the prepartum rise in foetal plasma cortisol concentration, there was a pronounced increase in foetal plasma ACTH concentration in the last 24 h of foetal life. In one of the two foetuses which initially had a low plasma ACTH concentration (681) ACTH rose to 882 pg ml⁻¹ in the last 6 h of intrauterine life. In the other foetus (686), ACTH rose to 1,680 pg ml⁻¹ 40 min before delivery. There was a further rise to 2,380 pg ml⁻¹ 5 min before delivery. Although strong, coordinated uterine contractions only occur in the last 6 h or so of gestation in the ewe, contractions are gradually increasing in frequency and amplitude over the last 24 h of foetal life. It may be that stimulation of the foetus by uterine contraction is one of the causes of the increase in plasma ACTH that occurs at this time. The ACTH released during this period is probably the cause of the rapid rise in cortisol concentration which occurs in the last few hours of foetal life. Immediately after delivery plasma ACTH reached 3,001 and 1,948 pg ml⁻¹ in two lambs (681 and 723).

We would emphasise that results which contain only single daily observations for foetal plasma ACTH must be viewed with caution for two reasons. First, it is well known that in the adult ACTH is secreted in an episodic manner¹⁸. The results obtained from the foetus which was sampled every 6 h showed that there were changes in both foetal plasma cortisol and ACTH concentrations during the day and there was some indication of a nyctohemeral rhythm in circulating concentrations. It may be that the total daily secretion of ACTH by the foetus increases before foetal cortisol rises. Such a mechanism would explain the increased sensitivity of the foetal adrenal which occurs just before parturition¹³, as well as the increased secretion of foetal corticosteroids^{6,13}. To investigate this possibility it will be necessary to sample the foetus more frequently. To avoid haemorrhagic stress an increase in the frequency of sampling will require the availability of the cytochemical assay for ACTH¹⁷. Second, in the adult mammal it is known that cortisol will exert a negative feedback on ACTH secretion at both physiological and pharmacological concentrations¹⁸. Thus, the high plasma corticosteroid concentrations immediately before delivery may be exerting a negative feedback action on ACTH secretion. The final level of ACTH secretion will depend on the summation of any such negative feedback and the positive drives on the hypothalamo-hypophyseal-adrenal axis.

In conclusion, simultaneous measurements of plasma ACTH and cortisol concentrations have been made on sequential blood samples obtained from seven chronically catheterised foetal sheep. Five of these foetuses were sampled sequentially for several days before delivery. The basal ACTH concentrations varied between animals. This variation in basal concentration cannot at present be explained. On the basis of the present data there was no evidence for an increase in foetal plasma ACTH before the preparturient rise in foetal plasma cortisol.

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Novel 4-hydroxycoumarin anticoagulants active against resistant rats

FIELD populations of common rats (*Rattus norvegicus*) resistant to anticoagulant rodenticides (for example, Warfarin) have appeared¹ in Scotland, Denmark, England and Wales, and more recently on the American continent. This resistance seems to be associated with a reduction in the efficiency of vitamin K metabolism¹ and is an inheritable factor controlled by a single dominant autosomal gene. Various theories have^{1,2} been advanced to explain the underlying mechanism. Resistance to other anticoagulants has now appeared, that to coumatetralyl being the least effective¹.

Reduction of the carbonyl group of Warfarin and replacement of the terminal methyl group with various substituted phenyl groups produces compounds showing increased anticoagulant activity^{4,5}. We have synthesised compounds of this type (compounds 1 and 2, Table 1) and have found that activity was only demonstrated in normal rats and not in the resistant strain.

Comparison of the structures of Warfarin and coumatetralyl suggested that the activity of the latter in resistant rats might be due to the manner in which, in the tetralin moiety, the four carbon atoms of the saturated ring are held adjacent to the unsaturated ring, whereas the four carbon atoms in the side chain of Warfarin can assume many conformations other than those adjacent to the benzene ring. Compounds were therefore synthesised containing substituted phenyl groups attached by a three carbon chain to a 4-hydroxy coumarin moiety, the three carbon chain being part of a saturated ring fused to a benzene ring. Examples of such are the 3-(3-*p*-substituted-1,2,3,4-tetrahydronaphth-1-yl) 4-hydroxycoumarins and related compounds shown in Table 1.

Several conclusions may be drawn from these results.

Table 1 The activity of 3-(3-*p*-substituted phenyl-1,2,3,4-tetrahydronaphth-1-yl)-4-hydroxycoumarins and related compounds in normal (Wistar) and homozygous resistant Welsh strain *Rattus norvegicus*

No.	Structure	Prothrombin ED ₅₀ mg kg ⁻¹		Resistance factor	Bait trial	
		Wistar	Resistant		Concentration (p.p.m.)	Mortality (%)
1		0.4	> 20	> 50	—	—
2		1.2	> 25	> 20	—	—
		1.2	25	20	—	—
	X R' R ²					
3	CH ₂ H 4-methyl	> 2.0	—	—	—	—
4	CH ₂ H 4- <i>n</i> -propyl	0.26	~0.8	~3	50	100
5	CH ₂ H 4-isopropyl	0.22	~0.8	~4	50	100
6	CH ₂ H 4-isobutyl	0.21	~0.6	~3	20	100
7	CH ₂ H 4- <i>n</i> -hexyl	0.20	~0.4	~2	50	100
8	CH ₂ H 4-dodecyl	0.28	~0.6	~2	50	100
9	CH ₂ H 4-cyclohexyl	0.33	~0.6	~2	50	100
10	CH ₂ H 4-phenyl	0.17	0.32	1.9	10	70
11	CH ₂ H 4-phenoxy	0.12	0.12	1.0	10	80
12	CH ₂ H 4-(<i>p</i> -chlorophenyl)	0.10	0.12	1.2	5	100
13	CH ₂ H 4-(<i>p</i> -bromophenyl)	0.08	0.10	1.2	2	80
14	CH ₂ H 4-(<i>p</i> -bromophenoxy)	0.07	0.07	1.0	1	40
15	CH ₂ H 4-chloro	0.09	0.14	1.6	5	100
16	CH ₂ H 4-bromo	0.13	0.22	1.7	5	100
17	CH ₂ H 4-benzyl	0.18	~0.3	~2	10	80
18	CH ₂ H 3,4-tetramethylene	> 5.0	—	—	—	—
19	CH ₂ Cl 4-phenyl	0.25	~0.5	~2	—	—
20	CH ₂ CH ₃ 4-phenyl	0.18	~0.4	~2	50	100
21	O H H	> 1.0	> 2.0	—	—	—
22	O H 4-phenyl	0.35	1.5	4.3	—	—
23	O H 4-chloro	0.42	~1.0	~2	—	—
24	O Cl 4-phenyl	0.26	~2.0	~8	—	—
25		0.31	~1.0	~3	20	20

Prothrombin ED₅₀: mg per kg of compound given in three daily doses predicted to increase prothrombin time (as measured by the one stage method of Quick) from a resting value of 16 s to 112 s on the fourth day. Male rats were used throughout. Diphacinone, chlorophacinone, coumatetralyl, S(−) Warfarin and R(+) Warfarin give prothrombin ED₅₀s of 0.22, 0.22, 0.31, 0.30 and 3.3 mg kg⁻¹, respectively in Wistar rats and of ~50.0, > 20.0, ~4.4, > 50 and > 50 mg kg⁻¹ respectively in resistant rats. Resistance factor: ratio of prothrombin ED₅₀s for resistant and normal rats. Diphacinone, chlorophacinone, coumatetralyl, S(−) Warfarin and R(+) Warfarin thus have factors of ~227, > 90, ~14, > 166 and > 15, respectively. Bait trial: rats were exposed to the test baits with no alternative food for 10 d; mortalities were recorded up to 14 d. Female Welsh strain homozygous resistant rats were used throughout. Diphacinone, S(−) and R(+) Warfarin did not cause death at 250 p.p.m. and chlorophacinone and coumatetralyl gave 20% and 40% mortality, respectively, at this concentration. The melting point of compound 1 was 200–201°C; satisfactory analytical data were obtained for the other compounds whose preparation will be described elsewhere. Compounds 3–24 are two component mixtures (thin layer chromatography, TLC) of what are believed to be *cis* and *trans* isomers. In two cases (compounds 10 and 12) the slower running isomer (TLC) was shown to be two to four times as active as the faster running isomer. Since the proportion of the slower running isomer in the mixtures is > 50% (TLC) this is unlikely to significantly alter the structure–activity relationships. Patent applications are pending on the 3-(3-*p*-substituted-phenyl-1,2,3,4-tetrahydronaphth-1-yl) 4-hydroxycoumarins.

The substituent in the *para* position of the phenyl group may vary in size with little effect on potency (compounds 4–8) although such limitations are indicated; for example, when the *para* substituent is methyl or tetralyl (compounds 3 and 18), anticoagulant activity is lost. A further aromatic ring joined either directly to the *para* position of the phenyl group (compound 10), or to the *para* position by oxygen or methylene bridges (compounds 11 and 17) seems to be advantageous. The most active compounds contain a *para* halogenophenyl group either joined directly to the *para* position of the phenyl group or joined to this position by an oxygen bridge (compounds 12–14).

A requirement for anticoagulant activity in either strain of rat seems to be a lipophilic group in the *para* position of the phenyl group, suggesting that the substituted phenyl group is providing a point of attachment to a lipophilic site to which Warfarin and other anticoagulants do not bond strongly. The high activity exhibited when the phenyl group contains *para* substituents with somewhat less lipophilic character (compounds 15 and 16) infers that electronic effects may also play some part. Variations in activity, however, do not parallel those reported for the series obtained by replacing the terminal methyl group of Warfarin alcohol by substituted phenyl groups⁴. Substitution in the 7 position of the tetralin ring reduces or has little effect upon activity (compounds 19 and 20). Replacement of the tetralin moiety by chroman or indanyl rings (compounds 21–25) reduces activity and increases resistance. The angle which the phenyl group makes with the indanyl group (compound 25) differs markedly from that made with the tetralin group (compounds 3–20); this may be responsible for the loss of activity.

Bait trials show good agreement with the prothrombin estimations, compounds with the lowest prothrombin ED₅₀ generally being the most active. The indications are that the new materials are well absorbed in the gut and are promising rodenticides. It is interesting to compare kills of resistant rats with those obtained at considerably higher concentrations with the standard anticoagulants (see notes to Table 1).

Tests with two of these compounds (10 and 13) indicate that they act in the same manner as Warfarin (M.R.H., unpublished) in that they are not classic competitive inhibitors of vitamin K₁. This contrasts with 2-chloro analogue of this vitamin which is also somewhat effective in resistant rats⁶, presumably because it affects a site other than that attacked by Warfarin and is not therefore involved in the resistance mechanism.

One of these compounds (10) has been tested in the field against wild rat infestations resistant to normal anticoagulants, providing control in baits containing only 0.005% (M.R.H. and B. D. Rennison, unpublished).

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Thermal transitions in collagen and the preferred temperature range of animals

We have shown^{1–3} that the melting point of the molecular collagen of both homoeothermic land poikilothermic animals correlates with the upper limit of their range of preferred temperatures (the range of temperatures they will voluntarily tolerate in their natural environment). We now report that for some poikilotherms the lower limit of the range of preferred temperatures also correlates with certain thermal properties of their collagen.

This conclusion was derived from an examination of the mechanical properties of earthworm (*Allolobophora caliginosa*) cuticle and jellyfish (*Aurelia coerulea*) connective tissue when these are heated in physiological saline (0.9% NaCl). Native tissues were used because the properties of collagen are best studied in a condition as close as possible to that in which they exist in nature. Such studies complement and indeed extend work involving purified and soluble collagens.

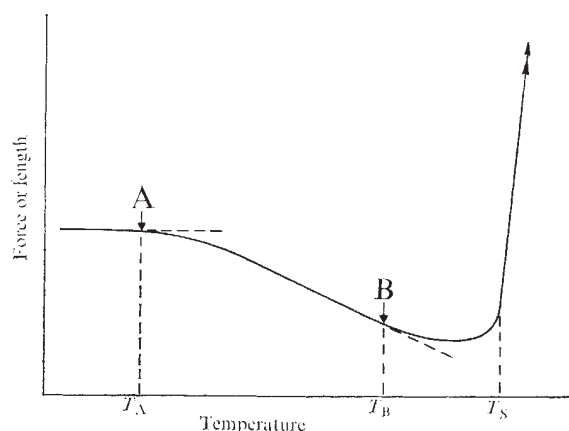
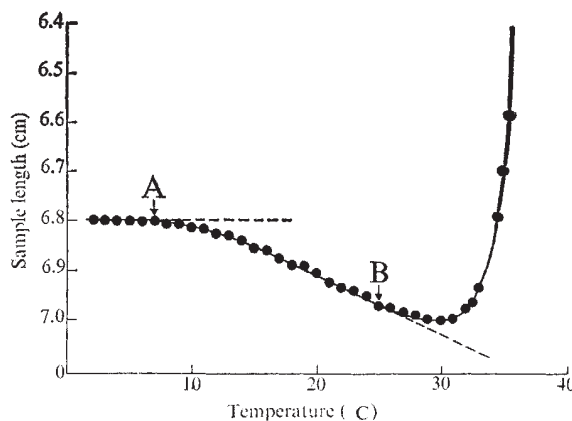


Fig. 1 Diagram defining thermal transitions in a sample immersed in 0.9% NaCl. The ordinate represents the property observed as temperature is increased. The transitions A and B are defined as points of deviation from linearity. T_S is the familiar shrinkage temperature; T_A and T_B are discussed in the text.

The cuticle of the earthworm was removed from an animal which had been killed by holding at 0°C overnight, and this cuticle formed an ideal sample (1–2 µm thick × 10 cm long) for direct length measurements as the temperature was varied. The ends of the specimen were clamped together at one end after looping through a glass ring of sufficient mass to just keep the sample straight. Length measurements were made

Fig. 2 A length–temperature curve for a freely suspended sample of the cuticle of earthworm *A. caliginosa* in NaCl. The transitions A and B are indicated by arrows at 7° and 25° C, respectively. T_S takes place at ~ 35° C.



with a cathetometer. As the applied stress was virtually zero, this experiment amounts to one-dimensional dilatometry.

The jellyfish samples were cut from the bell of the animal and stored in an acetone-water mixture until required. They were then washed and soaked in 0.9% NaCl overnight, by which time they had the appearance of fresh material. In the case of jellyfish samples it was not convenient to measure length, and the thermal behaviour was determined by observations of stress relaxation in the slightly extended specimen. One end of the specimen was held in a fixed clamp while the other was connected to a very stiff force transducer. Stress variation was recorded on a chart recorder. Ultimately, both methods detect molecular relaxation processes whether they be first or second order thermal transitions. The stress relaxation method, however, is somewhat more versatile as it is independent of sample dimensions.

Before beginning an experiment the samples were conditioned at the lowest experimental temperature for at least 16 h, or until, in the case of stress relaxation experiments, equilibrium was attained. Two heating rates were used: 0.1 or 0.5°C min⁻¹; but no difference could be detected between results obtained with either.

Table 1 Mean values for the three thermal transitions defined in Fig. 1 for the collagen of two earthworms and a jellyfish

Animal	No. of experiments	T_A	T_B	T_S	Preferred temperature range
<i>A. caliginosa</i>	10	8	23	35	10–23 (ref. 10)
<i>E. foetida</i>	6	12	23	35	16–23 (ref. 10)
<i>A. coerulea</i>	7	17	27	50	17–25 (ref. 3)

The range of preferred temperatures for the two earthworms reported by Grant¹⁰ are listed. In the case of the jellyfish, the range has been listed as that over which the pulsation rate of the bell is uniform and linear³, but is also close to the seawater temperature limits which the animal frequents.

Both experiments gave rise to a characteristic curve (Fig. 1). There is always a linear region in which there is little change in length or force with temperature. The first transition occurs at A, then a less marked transition at B, and finally the sample shrinks. The temperatures at which the three transitions take place are denoted by T_A , T_B and T_S . At T_S , the familiar shrinkage temperature, the length decreases for the freely suspended sample and force increases in the stress relaxation experiment, because the sample is constrained. As long as the temperature is not allowed to exceed T_B by more than 2–3°C the sample may be cooled to below T_A and the cycle repeated. In other words the transitions are reversible. In Figs 2 and 3 the dotted lines are drawn to show more clearly the transition at A and B. The mean values for the transitions in all experiments, including another species of earthworm *Eisenia foetida* are given in Table 1.

A full description and interpretation of these transitions will be given elsewhere. Here we are interested only in the biological significance of the transitions, especially those occurring at A and B. We have already said that T_S marks the region of the well known thermal shrinkage phenomenon, which has been shown to be a manifestation of the melting of crystalline collagen^{4,5}.

The mean temperature of the transition at B of the collagen of *A. caliginosa*,^{1,6} and *A. coerulea*³ agrees with the temperature at which the molecular collagen melts in dilute solution (that is, T_D , the mid-point of the transition as determined by optical rotation or viscometry), or what we have shown to be equivalent—the temperature at which these tissues shrink when heated in HCl solution at pH 1 (ref. 7). Determination of T_D for the collagen of *E. foetida* in HCl gave 22°C, which again is close to T_B . A number of other earthworms^{1,6} have a T_D value of

22°C, and Maser and Rice⁸ and Josse and Harrington⁹ using optical rotation report 22°C for another earthworm *Lumbricus terrestris*. So, we think it justifiable to equate the tissue transition T_B with T_D , the molecular transition.

Before commenting on the transition at A we need to consider the following. The molecular melting temperature, T_D , of the collagen of a wide range of animals agrees with the upper limit of their preferred thermal range, if they are poikilothermic, or with their normal body temperature if they are homeothermic^{1–3}. Now, in view of the preceding paragraph, when dealing with tissues, we can say that T_B correlates in the same way. When we found the transition at A, we wondered if it had any relation to the lower limit of the preferendum. This turned out to be the case on examining the literature. First, our own data³ for the jellyfish *A. coerulea* showed that: over the range 17°–26°C the pulsation rate of the bell of a live animal was uniform and linear with temperature; at 15°C the jellyfish had become sluggish, while above 28°C the pulsation rate dropped very rapidly. Furthermore, the mean limits of surface water temperature along the New South Wales coast where the animal was taken, are 17°–23°C (ref. 3).

Although we originally linked the upper value of 26°C (as determined by the pulsation behaviour of the bell) with the value of the melting temperature of its collagen, we did not look for evidence of a structural transition near the lower limit of 17°C. These data have been incorporated into Fig. 3, and an examination of the figure shows that thermal transitions A and B (curve 1) are very close to the lower and upper limits of the normal pulsation rates of the jellyfish respectively

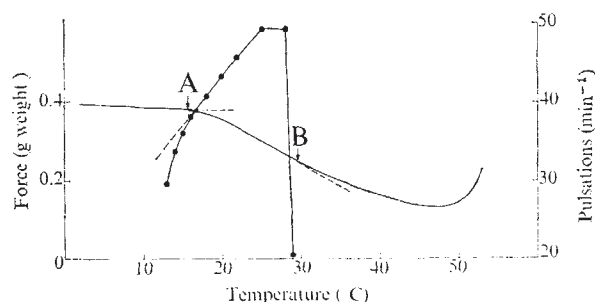


Fig. 3 A stress relaxation-temperature curve for jellyfish connective tissue in 0.9% NaCl, together with data (●) showing the pulsation rate of the bell plotted against temperature for a live jellyfish in seawater³. The species was *A. coerulea*.

(curve 2), and both points correspond well with the limits of its range of preferred temperatures.³ Second, in the case of the earthworm it has been reported by Grant¹⁰, that *A. caliginosa* and *E. foetida* have thermal preference ranges of 10°–23°C and 16°–23°C respectively. The upper limits agree very well with T_B in Table 1, and the lower limits are close to T_A for each species.

We suggest that transition temperature T_A , marks the lower limit of the animal's range of preferred temperatures just as T_B (or, T_D in dilute solution studies on molecular collagen) marks the upper limit. Further, it seems that transition A represents the beginning of a disorganisation of molecular collagen, but that complete melting of the molecule in dilute solution will not take place until a temperature greater than T_B is reached. Whether the properties of collagen determine the range of preferred temperatures or vice versa, is an open question.

When we compare dilute solution studies with tissue studies only T_D and T_B are coincident. There is no evidence in the work of either Maser and Rice⁸ or of Josse and Harrington⁹ of a transition at a temperature corresponding to T_A . This may be because they used a different species of earthworm; or because the use of native tissue preserves non-collagenous components

which give rise to the transition at A, or possibly, there are other collagens with lower thermal stabilities, which have been destroyed by the usual solubilisation procedure.

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Reduced maximal levels of derepression of the isoleucine–valine and leucine enzymes in *hisT* mutants of *Salmonella typhimurium*

STRAINS of *Salmonella typhimurium* with a mutation in the *hisT* locus, produce an enzyme that cannot convert two uridine residues to pseudouridine in the anti-codon region of tRNA^{His} (refs 1 and 2). This mutation allows constitutive synthesis of the histidine biosynthetic enzymes^{1,3}. Recently, *hisT* mutants were found to have changes in tRNA^{Leu} (refs 1, 4 and 5), in tRNA^{Ile} (ref. 6), and to be partially derepressed for the isoleucine–valine biosynthetic enzymes^{5,6}. This suggests strongly that tRNA is part of the repression machinery for these enzymes. We report here that *hisT* strains are greatly limited in their ability to derepress the isoleucine–valine and leucine enzymes during leucine starvation. Thus, the *hisT* mutation alters regulation of these enzymes in two ways; it seems to cause a marked reduction in their maximal level as well as leading to a partial loss of repressibility.

A leucine-requiring strain of *S. typhimurium* containing the *hisT1504* lesion was constructed by transduction with phage P22. When the strain was grown in the presence of excess branched-chain amino acids, the level of the isoleucine valine and leucine enzymes was essentially the same as that found for the prototrophic *hisT1504* parent strain (Table 1). During leucine starvation these enzymes were increased two- to three-fold in the strain containing both *hisT* and *leu* mutations, while the isogenic strain without the *hisT* mutation was derepressed 14- to 116-fold when grown under the same conditions (Table 1). This effect seems specific for the isoleucine–valine and leucine enzymes since the levels of two unrelated enzymes, hexokinase and acetylornithinase, are equivalent in both strains during leucine restriction (M.F. unpublished).

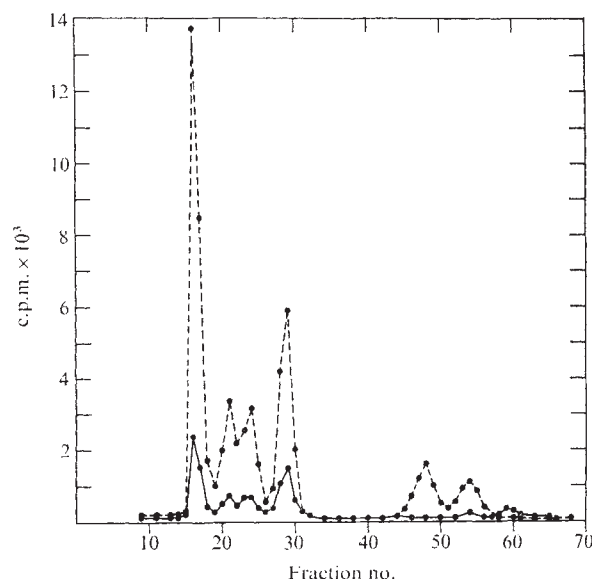


Fig. 1 *In vivo* levels of charged tRNA^{Leu} in the *hisT* strain grown on limiting leucine. The cells were grown in minimal medium with L-isoleucine (50 µg ml⁻¹), L-valine (100 µg ml⁻¹) and L-leucine (9 µg ml⁻¹). The tRNA was isolated as described previously⁵ except that after phenol extraction and centrifugation, the aqueous phase was layered on to a DEAE-cellulose column equilibrated with 0.3 M LiCl in 0.1 M sodium acetate buffer, pH 5.0. The column was then washed with several bed volumes of this buffer. The tRNA was eluted with 1.0 M LiCl and precipitated with 2.5 volumes of ice-cold ethanol. The isolated tRNA was divided into two portions; one was kept as control and the second sample was oxidised with sodium periodate as described previously⁵. The control and periodated samples were charged with ³H-leucine and ¹⁴C-leucine respectively and mixed and cochromatographed on an RPC-5 reversed-phase column as described previously⁵. The altered mobility of the last two isoacceptor species from the periodated sample is an artefact of the periodate oxidation procedure⁵ (A.A.R. and M.F., unpublished). ● — ●, ³H unperiodated; ● — ●, ¹⁴C periodated.

To investigate this further, both strains were grown with various amounts of leucine. The levels of the isoleucine–valine and leucine enzymes in the strain with the *hisT* mutation did not change more than threefold for the entire range of leucine concentrations (Table 2). There was a strong inverse relationship between the amount of leucine and the enzyme levels found in the isogenic strain. Growth of these strains in a chemostat with a limiting supply of leucine produced similar results (Table 2). Under all conditions tested the *hisT* mutation seems to have curtailed the range of derepression during leucine limitation as well as reducing the maximal level of the isoleucine–valine and leucine enzymes that can be made under these conditions.

The limited ability of strains with a *hisT* mutation to derepress is not common to all strains that show partial constitutive

Table 1 Effect of the *hisT* mutation on derepression of the isoleucine–valine and leucine enzymes and levels of charged tRNA

Mutation	Growth conditions	Threonine deaminase	Specific activity			tRNA charged <i>in vivo</i> (%)	
			Acetohydroxy acid synthetase	β-IPMD		Leucine	Valine
<i>leu124</i>	Excess leucine	8.2	0.7	1.7		82	90
<i>leu124</i>	Limiting leucine	107.6	82.3	91.2		27	87
<i>hisT1504 leu124</i>	Excess leucine	21.2	7.6	12.4		91	88
<i>hisT1504 leu124</i>	Limiting leucine	33.6	18.9	20.3		18	92

All bacterial strains were derived from *S. typhimurium* LT-2. The strain containing the *hisT1504* mutations was constructed by P22 transduction⁷. The *hisT* allele was transduced into a strain containing the mutations *aroD38 leu124*, selecting for Aro⁺ recombinants. The *hisT* lesion was identified by colony morphology⁸, growth rate⁹, tRNA^{Leu} profile^{1,5} and enzyme levels^{5,6}. The minimal medium and growth conditions were as described previously⁵. The minimal medium was supplemented with L-isoleucine (50 µg ml⁻¹), L-valine (100 µg ml⁻¹) and excess L-leucine (50 µg ml⁻¹) or limiting L-leucine (7.5 µg ml⁻¹). Enzyme specific activity is expressed as µmol of product per mg of protein per hour. The preparation of the crude cell extracts and the measurement of enzyme activity and protein was as described previously⁵. The leucine enzyme measured in this study was β-isopropylmalate dehydrogenase (β-IPMD). Isolation and periodate oxidation and the percentage of tRNA that existed in the charged form *in vivo* were as described previously⁵.

Table 2 Effect of the *hisT* mutation on derepression during growth limitation on various concentrations of leucine

Mutation*	L-Leucine ($\mu\text{g ml}^{-1}$)	Specific activity	
		Threonine deaminase	Acetohydroxy acid synthetase
<i>leu124</i>	7.5 chemostat†	123.2	82.5
<i>leu124</i>	7.5	101.1	61.2
<i>leu124</i>	12.5	59.9	45.0
<i>leu124</i>	15	53.3	19.4
<i>leu124</i>	17.5	42.3	16.0
<i>leu124</i>	20	27.7	8.3
<i>leu124</i>	50	5.6	1.0
<i>hisTleu124</i>	7.5 chemostat	51.0	25.0
<i>hisTleu124</i>	7.5	41.0	26.1
<i>hisTleu124</i>	12.5	30.0	10.8
<i>hisTleu124</i>	15	28.5	10.8
<i>hisTleu124</i>	17.5	29.6	10.6
<i>hisTleu124</i>	20	27.4	7.9
<i>f1rB1leu124</i>	7.5	138.0	118.6
<i>f1rB1leu124</i>	50	63.8	77.0

*The strain containing the *f1rB1leu124* mutations was constructed by transducing the *leu124* mutation into a strain containing the mutations *ara123*, selecting for *Ara*⁺ recombinants. *f1rB* strains are resistant to the leucine analogue 5',5',5'-trifluoroleucine and are constitutive for enzymes forming branched-chain amino acids. The biochemical basis for this phenotype is unknown¹⁰.

†In experiments with a chemostat the minimal medium was supplemented with L-isoleucine (50 $\mu\text{g ml}^{-1}$), L-valine (100 $\mu\text{g ml}^{-1}$) and L-leucine (7.5 $\mu\text{g ml}^{-1}$). Enzymes were measured after four doublings. All other experiments involved Erlenmeyer flasks for measurements of growth and enzyme levels. The minimal medium contained L-isoleucine, L-valine and L-leucine, as indicated. In all experiments the doubling time of the *hisT* mutant was 30–35% less than the isogenic strain. This reduced growth rate is about the same as reported for *hisT* strains prototrophic for leucine, grown in minimal medium⁴. For all other conditions and explanations see Table 1.

synthesis of the enzymes. A strain with a *f1rB1* mutation is highly rederepressed for the isoleucine–valine and leucine enzymes due to a mutation other than *hisT* (ref. 10). We transduced the same *leu* mutation into this strain as we had for the *hisT1504* mutant. Derepression of the isoleucine–valine and leucine enzymes during leucine starvation was greater in this mutant than in the isogenic leucine-requiring strain and three to five times greater than for the *hisT* leucine auxotroph (Table 2).

A direct correlation has been found between decreased levels of charged tRNA^{Leu} and derepression of the isoleucine–valine and leucine enzymes¹¹. The difference in derepression found in the strain with the *hisT* mutation might therefore be due to variations in tRNA^{Leu} acylation during leucine starvation. This does not seem to be the case since bulk tRNA^{Leu} was about equally deacylated in both strains when they were starved for leucine (Table 1). In addition, the *in vivo* charging of the individual species of tRNA^{Leu} was examined by the reversed-phase chromatography system (RPC-5) of Kelmers and Heatherly¹². All components of leucine-acceptor activity were highly deacylated when the strain with the *hisT* lesion was grown on limiting amounts of leucine (Fig. 1).

Previous work has established that tRNA^{Leu} is altered in *hisT* mutants and that these strains are partially derepressed for the isoleucine–valine and leucine enzymes^{1,5,6}. In addition, our results suggest that the *hisT* mutation alters further the repression mechanism in these pathways by reducing their maximal expression during leucine limitation. These data offer additional evidence for the involvement of tRNA^{Leu} in repression of the isoleucine–valine and leucine enzymes. The dual effect of the *hisT* mutation on the regulation of these enzymes suggests that the lack of pseudouridine in the anticodon region of tRNA^{Leu} introduces a conformational change in the tRNA which affects both minimal and enzyme levels. Thus, the conformational change which may normally occur in tRNA^{Leu} during aminoacylation to cause repression may be defective in tRNA^{Leu} from *hisT* mutants. In addition, this conformation may elicit partial repression even if the tRNA is deacylated. Alternatively, a species of tRNA^{Leu} may have a positive role in regulation. The alteration of such a species in *hisT* mutants could prevent

the full expression of the isoleucine–valine and leucine enzymes. Because of the pleiotropic nature of the *hisT* mutation^{1,2}, it is possible that another altered tRNA rather than, or in addition to tRNA^{Leu} is responsible for these changes in repression. Cortese *et al.*⁶ reported a possible alteration in a minor species of tRNA^{Leu} in *hisT* mutants. Changes in tRNA^{Leu} do not, however, seem to alter the regulation in these systems since the form of acetohydroxy acid synthetase that is not subject to feedback inhibition by valine is not disproportionately elevated in *hisT* strains (unpublished observations of M. F.). It has been shown that due to an alteration in the isoleucine 'repression signal' the resistant but not the valine-sensitive form of acetohydroxy acid synthetase is derepressed^{13,14}. It is also conceivable that the encoded product of the *hisT* gene could be involved in regulation of the isoleucine–valine and leucine enzymes. Utilisation of *hisT* mutants in an *in vitro* protein synthesising system will aid the choice from among these possibilities.

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Chromosome-21-dosage effect on inducibility of anti-viral gene(s)

UNLIKE the bacterial situation little is known about gene regulation in mammalian cells. Inducible systems are being used to study regulatory gene functions in normal and abnormal mammalian cells^{1–3}, and have shown for example, that inhibitors of macromolecular synthesis enhance rather than inhibit the expression of an inducible enzyme. Actinomycin D enhances the induction of tyrosine aminotransferase (TAT) by steroid hormones in hepatoma cells⁴. Commonly referred to as superinduction, this effect has led to the hypothesis that a regulatory gene(s) modulates the expression of the structural TAT gene¹. Of course, alternative explanations have been offered for the superinducing effect of actinomycin D (refs 5 and 6). Similarly, the induction of interferon by viruses and poly(I)·poly(C) and the induction of the anti-viral state (AVS) by interferon have been used to probe regulatory mechanisms in normal mammalian cells^{7–13}. It has been demonstrated that with judicious use of metabolic inhibitors the amount of interferon and the level of AVS induced can be enhanced 100–1,000 times and 10–100 times respectively, over that obtained in cultures exposed to inducer alone. This suggested that one or more regulatory gene(s) modulate the expression of the structural gene for interferon and AVS^{7–19}.

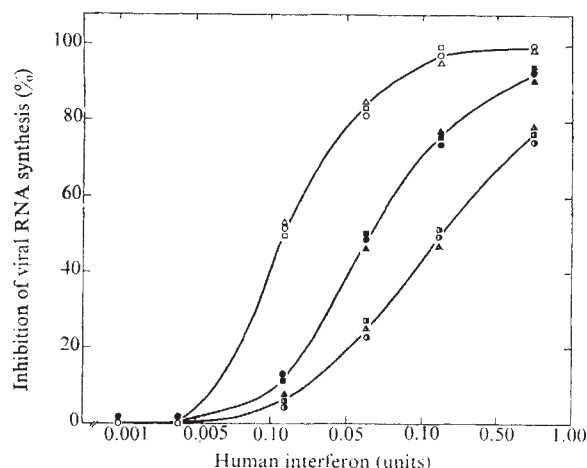


Fig. 1 Induction of the AVS in human skin fibroblasts monosomic (cell line 198 halved symbols), disomic (cell line 141 closed symbols), and trisomic (cell line 258, open symbols) for chromosome 21 by human interferon. Three sets of experiments were performed for each cell line described.

I have used a new approach to assess the presence of a regulatory gene function which modulates the expression of the structural gene for AVS. I found evidence to suggest that the regulation of the anti-viral genes in human cells is controlled by regulatory gene element(s) located on a separate chromosome.

AVS was induced in human fibroblast cultures trisomic (T-21) disomic (D-21) and monosomic (M-21) for chromosome 21, trisomic for chromosome 18 (T-18), monosomic for chromosome 5 (M-5) and carrying a partial deletion of chromosome 4 (M-4) by exposing them to various concentrations of human interferon. The level of AVS induced was estimated by assessing the amount of interferon required to inhibit viral RNA synthesis by 50%. Figure 1 and Table 1 show that M-21 fibroblasts were less sensitive to the anti-viral action of interferon than normal diploid fibroblasts, which were in turn less sensitive than T-21 fibroblasts. The concentration of interferon required to induce AVS in M-4, M-5 and T-18 fibroblasts falls within the same range of concentrations required to induce AVS in normal diploid fibroblasts, suggesting that the effect of

Table 1 Induction of AVS in human skin fibroblasts

Cell type	Identification no.	Units human interferon inhibiting vital RNA synthesis by 50%
Monosomic 21	GM-137	0.24, 0.24, 0.27
	GM-230	0.26, 0.29
	198	0.30, 0.24, 0.25, 0.24
Disomic 21	GM-37	0.060, 0.064
	GM-181	0.055, 0.061
	141	0.062, 0.064, 0.059, 0.069
Trisomic 21	GM-258	0.0157, 0.0157, 0.0156, 0.0180
	DS-GG1	0.0157, 0.0172
	DS-GG2	0.0160, 0.0157
Partially deleted 5	GM-72	0.060, 0.065
Monosomic 5	GM-71	0.062, 0.059, 0.060
Trisomic 18	(Ref. 21)	0.059, 0.070

Human fibroblast cultures established from skin biopsies were provided by the Mammalian Genetic Mutant Cell Repository, Camden, New Jersey, the Repository for Mutant Human Cell strains, Montreal and by Drs J. Mahoney and K. Halloran of Yale University. Three human fibroblast cultures trisomic for chromosome 21 (T-21) designated GM-258, DS-GG1 and DS-GG2; three human cultures monosomic for chromosome 21 (M-21) designated GM-137, GM-230 and 198; three normal diploid human skin fibroblasts, disomic for chromosome 21 (D-21) designated GM-37, GM-181 and 141, and two human fibroblast cultures, one known to carry a partially deleted chromosome 4 and the other known to have one less chromosome 5 designated GM-72 and GM-71 respectively were used. A trisomic 18 human fibroblast (T-18) derived from a previous study²⁷ was also used as one of the controls for the trisomic 21 cultures. The monosomic 21 lines used were verified by the Mammalian Genetic Mutant Cell Repository to contain 45 human chromosomes and to lack one chromosome 21. No translocation was evident. The amount of vesicular stomatitis virus replication was assayed by Skehan's method²⁸ except that (a) the cultures were exposed to actinomycin D ($4 \mu\text{g ml}^{-1}$) for 1 h before the viral challenge and (b) the cultures were incubated with the virus for 8 h instead of 14–17 h at 37°C . This modification of the procedure was introduced to reduce variability in the estimation of viral RNA synthesis. The results listed for the same cell type were derived from the fibroblasts at early cell passages. The interferon used in this study was derived from a large batch of human interferon that was contributed by Dr J. Valenta of Smith, Kline and French Pharmaceutical. This was purified through two cycles of gel filtration on Sephadex G-75 columns. The purified interferon had a specific activity of $\sim 1 \times 10^5 \text{ U mg}^{-1}$ protein. The unit of interferon described here is based on the internal standard of Smith, Kline and French interferon.

chromosome 21 on AVS is not a generalised effect resulting from the loss or gain of a human chromosome.

Figure 1 and Table 1 also show that the inducibility of the AVS in human cells increases nonlinearly for each increment in the number of human chromosome 21 present. To analyse this effect, the mean concentration of human interferon required to induce the AVS in fibroblasts containing one, two or three chromosome 21 (Table 1) was plotted logarithmically against the number of chromosome 21. A straight line was obtained which can be expressed by the equation

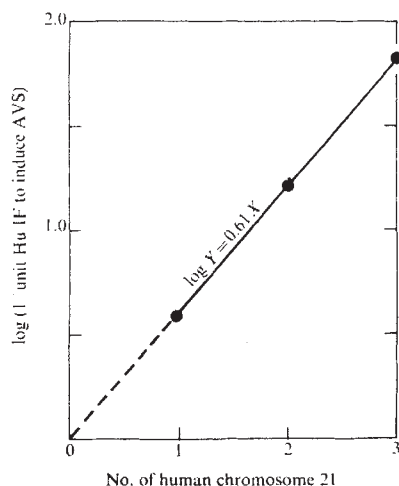
$$\log Y = 0.61X$$

where Y denotes the inducibility of AVS and X the number of chromosome 21 (Fig. 2). Thus, the inducibility of AVS increases logarithmically as the number of chromosome 21 increases linearly. Extrapolation of the curve to the origin suggests that a cell cannot be induced for AVS if chromosome 21 is absent. This confirms the assignment of the AVS gene(s) to chromosome 21 (ref. 18).

Before attempting to interpret the significance of this logarithmic gene dosage effect, it is useful to consider events that might contribute to the establishment of the AVS. They are (1) the uptake of interferon by its receptor sites; (2) the depression of the structural gene(s) which code(s) for AVS by an inducer, presumably interferon; (3) the transformation of cells into an AVS through production of an anti-viral protein; and (4) the modulation of the level of the AVS by regulatory gene element(s).

The existence of interferon receptor sites has not been substantiated. The existence of the structural gene(s) described in step (2) is based on genetic analysis of somatic cell hybrids derived from hamster $\times$ mouse, monkey $\times$ mouse

Fig. 2 Gene dosage effect of chromosome 21 on the induction of the AVS in human fibroblasts monosomic, disomic and trisomic for chromosome 21. Each point represents the mean reciprocal concentration of human interferon (HuIF) required to induce the AVS in the fibroblasts. The mean concentration of interferon is derived from the figures given in Table 1.



and human $\times$ mouse¹⁷⁻²⁰. This has led to the assignment of the gene(s) which codes for AVS and the cytoplasmic form of superoxide dismutase to chromosome 21 in man¹⁸.

The mechanisms of transformation of a cell to an AVS in step (3) is unclear, though it is presumed to be mediated by a protein²¹ which inhibits the translation of exogenous mRNA²²⁻²⁶. Conceivably the chromosome-21-directed AVS gene(s) produces this inhibitor substance. I have already discussed the existence of the regulatory gene function in step (4).

Accordingly, any increase in the number of chromosome 21 should be followed by a linear and proportional increase in the amount of chromosome-21-directed gene products. Instead my data show a logarithmic increase, showing that other complex mechanisms, presumably of a regulatory nature are involved. One such mechanism is that (1) the regulatory gene element(s) postulated by Chany *et al.*¹⁰ or (2) the repressor substances which normally maintain the anti-viral gene(s) in a non-constitutive state, is not located on chromosome 21. The effect of an increase in chromosome 21 would be an imbalance in the ratio of the AVS gene(s) and its regulator gene element(s) which is postulated to be located on a chromosome other than 21. This imbalance could mitigate the normal role of the regulator gene(s) in controlling the levels of AVS directed by the AVS gene(s).

The logarithmic increase in the amount of chromosome-21-directed product as the number of chromosome 21 increases linearly indicates that cellular induction of AVS can be mediated through interchromosomal gene interactions. This can be tested, for example, using an interferon selective system on a population of aneuploid human cells to select for cells expressing high and low levels of AVS. The levels of AVS in turn are determined by chromosome 21 dosage and dosage of the chromosome(s) other than chromosome 21 carrying the regulator gene element(s) for AVS. In any event, it is possible that cellular regulation by interchromosomal gene interaction is responsible for human disorders associated with the loss or gain of a single human chromosome.

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Multiple conformations of ribosomal precursor RNA

WE wish to propose an explanation for the heterogeneity shown¹ by ribosomal precursor RNAs on gel electrophoresis. The clearest demonstration of this phenomenon is shown in Fig. 1. ³²P-labelled RNA was eluted from single gel slices taken across the peak, and rerun in the presence of ³H-labelled marker. The ³²P-RNA samples run in their original positions and not coincidentally with the marker, that is, the width of the peak does not result from diffusion alone.

A heterogeneity of the 45S precursor of L cells has also

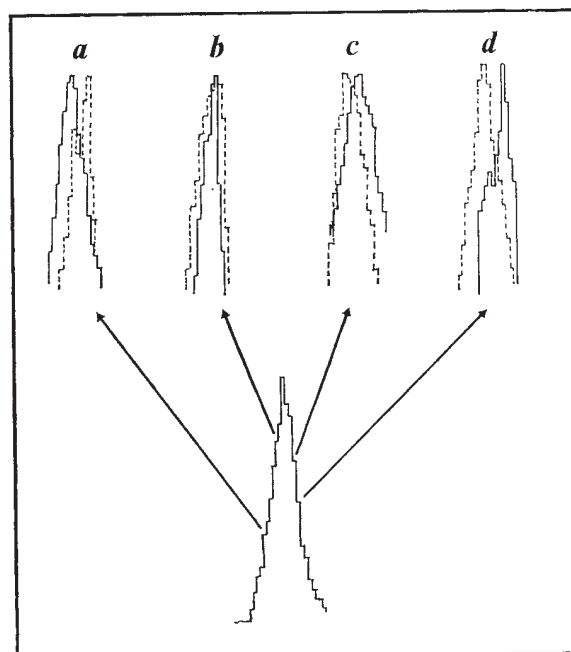


Fig. 1 The heterogeneity effect. The lower histogram represents ³²P-radioactivity in 0.5 mm slices from the 40S peak of *Xenopus* RNA run on a 2.4% polyacrylamide gel. RNA was eluted from individual gel slices by the method described¹. Each sample was mixed with 40S RNA labelled with ³H-uridine and rerun on a second 2.4% gel (upper histograms a-d). These gels were sliced to 0.5 mm and the slices were counted in scintillator. ---, ³H-Radioactivity; —, ³²P-radioactivity. The methods used to label the cells, extract the RNA, run, slice and count the gels have been described^{2,3}.

been reported, which was demonstrated by the effect of the radioactive pulse length on the gel mobility⁴. Although mobility in polyacrylamide gels depends mainly on molecular weight, it is known that other factors can have an effect⁵. Three possible explanations for the phenomenon may be proposed, although they are not necessarily mutually exclusive: (1) The original RNA peak is actually a mixture of components differing in length, perhaps because of processing reactions; (2) heterogeneity in primary sequence, because of divergence between the multiple ribosomal gene copies, is sufficient to cause a heterogeneity in gel mobility; and (3) the heterogeneity is caused by differences in secondary structure between otherwise identical molecules.

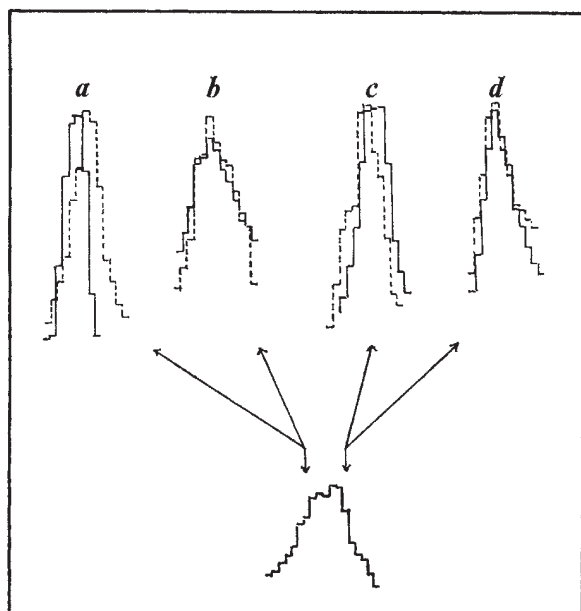
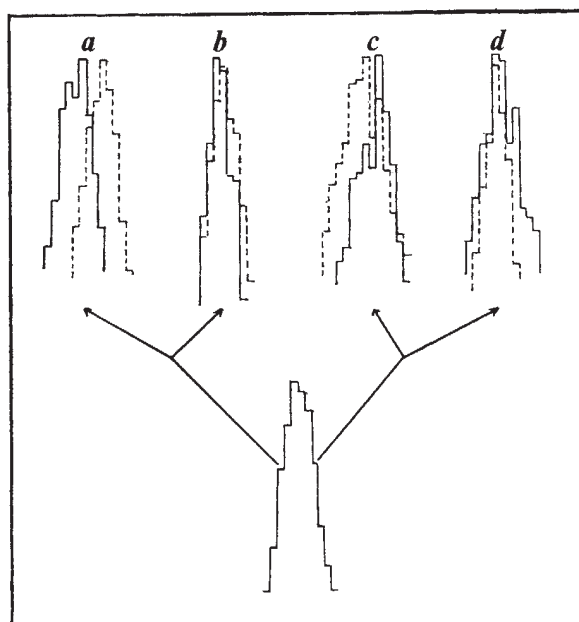


Fig. 2 Abolition of heterogeneity by formamide treatment of *Xenopus* 40S RNA. ^{32}P -labelled RNA from the indicated slices was mixed in E buffer² with total cell RNA pulse labelled with ^3H -uridine. The mixtures were divided, half being run as controls (a and c) and the other half (b and d) being treated before running with 70% formamide at 37° C for 5 min. The formamide was deionised by passage through mixed Amberlites IR-120/IRA-400. All glassware and solutions were sterilised with a dilute solution of diethyl pyrocarbonate. -----, ^3H -Radioactivity; ———, ^{32}P -radioactivity.

Figures 2 and 3 show the effect of partial denaturation of the eluted RNA fraction (plus marker) using heat treatment or formamide. Figure 2 shows the 40S *Xenopus* precursor^{3,6} and Fig. 3 the 42S precursor from yeast^{7,8}, in our case *Schizosaccharomyces pombe*. In both experiments the heterogeneity has been abolished by the treatment, thus ruling out the first of the possible explanations. In addition,

Fig. 3 Abolition of heterogeneity by heat treatment of yeast 42S RNA. The experimental design was the same as that of Fig. 2 except that the treatment consisted of heating to 60° C for 5 min. -----, ^3H -Radioactivity; ———, ^{32}P -radioactivity.



the 40S RNA has a unique 5' terminal nucleotide⁹. The second explanation could still be correct insofar as any primary sequence divergence might manifest itself as secondary structure differences. If it could be shown that the RNA reanneals during the period for which the gel is run, usually 4 h, this would be unlikely. It should be stressed that the formamide treatment lasts for 5 min only and the gels are normal ones, run in an aqueous medium rather than the denaturing formamide medium described by Staynov *et al.*¹⁰.

The total RNA preparation was treated with formamide and the RNA from single slices of the precursor peak rerun with marker. The heterogeneity is evident from Fig. 4, indicating that the RNA does reanneal within the gel. We would not, therefore, expect the experimental procedure of Figs 2 and 3 to remove heterogeneity caused by the effect of primary structure divergence on secondary structure. Furthermore, since heterogeneity can reappear after treatment of the total RNA, it would seem that the relevant secondary structure differences are characteristic of the molecules themselves rather than of other influences *in vivo*. For example, ribosomal proteins can affect the conformation adopted by ribosomal RNA¹¹.

The most likely interpretation of these experiments is that several secondary structure configurations are accessible to the molecules but that they do not readily interconvert once formed. Unless there is something peculiar

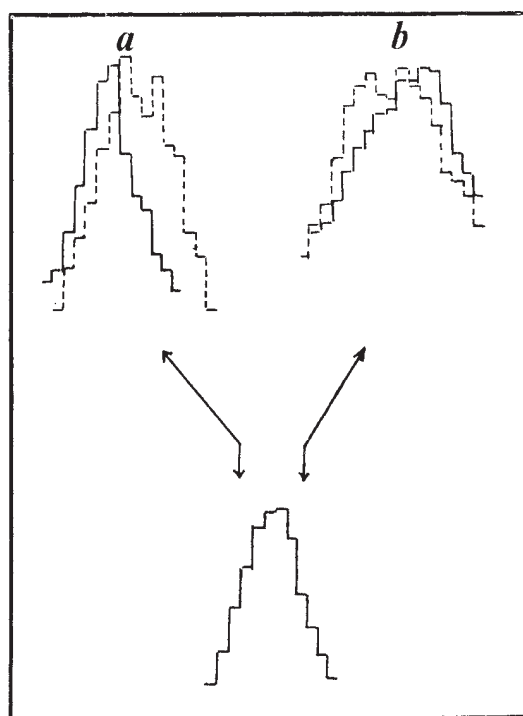


Fig. 4 Retention of heterogeneity after formamide treatment of the ^{32}P -labelled total cell RNA before running it on the first gel. -----, ^3H -Radioactivity; ———, ^{32}P -radioactivity.

about ribosomal precursor RNAs this suggests that other high molecular weight RNAs should show the same effect, and in fact we have found a small heterogeneity *Xenopus* 28S RNA and yeast 18S RNA which is not found after partial denaturation.

An RNA molecule is usually presumed to have a definite secondary structure which is the most stable arrangement of its primary sequence in antiparallel A-U and C-G base paired loops^{12,13}. The most stable arrangement may not be the same in different chemical environments; for

example, denatured forms of transfer and 5S RNA are known which are produced by the action of urea and EDTA^{14,15}, and in the case of 5S RNA the two forms are known to have different arrangements of the base paired regions¹⁶.

What our experiments suggest is that a collection of identical molecules placed in the same environment can spontaneously attain a range of different secondary structures. Structures may be regarded as different when the activation energy for interconversion is too high for rapid equilibration to occur (by comparison the activation energy for 5S renaturation has been found to be about 65 kcal/mol¹⁷). We would expect this property to be more evident for the high molecular weight RNAs because the greater length of the sequence should allow more base pairing arrangements, and indeed the low molecular weight RNAs isolated with modern techniques seem to be homogeneous in their three-dimensional structure, as evidenced, for example, by the crystallisation of tRNA^{18,19}.

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Crystal structure of proflavine, a DNA binding agent

THE subject of DNA-small molecule binding has received much attention in recent years. Particular emphasis has been placed on aminoacridine binding¹, and many studies have employed proflavine (Fig. 1) as perhaps the classical interacting agent of these series of drugs. The DNA-binding properties of proflavine cause it to act as a frameshift mutagen, and this ability has been extensively utilised in molecular genetics².

Proflavine has been shown to bind to DNA in two ways^{1,3}. First a strong interaction at low drug concentrations, involving intercalation of the planar drug molecule between successive base pairs⁴, and second a weaker process at higher drug levels primarily concerned with interactions with the phosphates external to the DNA double helix, although the precise stereochemical details of both the binding modes are not clear. It has been suggested, in a modification to the original intercalation model, that strong binding also involves ring

nitrogen-phosphate interactions⁵. As part of our studies directed to understanding drug-DNA binding, we have determined by single-crystal X-ray analysis the structure of proflavine itself, as the biologically active hemi-sulphate.

Proflavine hemi-sulphate crystallises from aqueous solution as well-formed, deep-red elongated prisms, belonging to the monoclinic space group $P2_1/c$. The cell dimensions are $a=12.703(1)$, $b=19.940(2)$, $c=21.487(2)$ Å, and $\beta=92.24(1)$ Å. This surprisingly large unit cell, together with measurements of crystal density, suggested that there were several proflavine

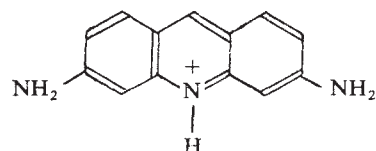


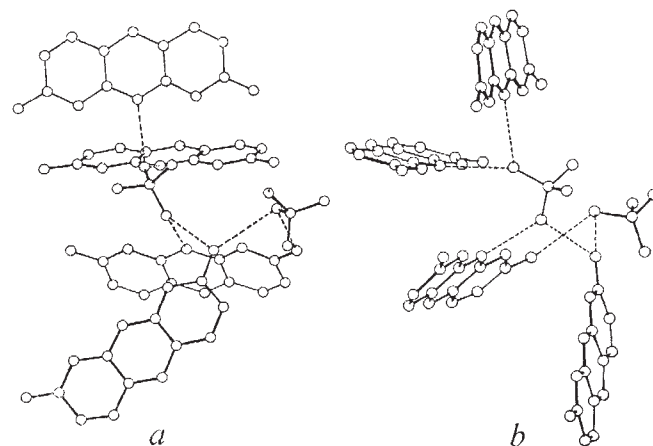
Fig. 1 Proflavine.

molecules in the crystallographic asymmetric unit, and this has been confirmed by our structure analysis. A total of 5,600 independent reflection intensities were measured on an automatic four-circle diffractometer.

The structure was solved by a combination of direct methods⁶ and reciprocal-space search procedures⁷, and refined by least-squares methods to a final discrepancy index of 0.0736. (The full details of the structure solution, as well as other crystallographic discussion, will be published elsewhere.) All the hydrogen atoms were located, except those attached to water molecules. As suggested by Albert⁸, the cationic charge of the proflavine rings has been found to reside exclusively on the central nitrogen atom, as evidenced by the location of a hydrogen atom attached to this atom, and coplanar with the ring. Our analysis has revealed that the asymmetric unit consists of four proflavine molecules, together with two sulphate ions and seven water molecules, a total of 81 independent non-hydrogen atoms. These four proflavine molecules are not related by any local symmetry elements.

The crystal structure has a number of features of interest. As might be expected, the lattice is held together by a complex network of hydrogen-bonding and electrostatic interactions. Figure 2 shows one unit of four proflavines, and illustrates their peculiar clustering around the sulphate ions, with one of

Fig. 2 *a* and *b* two views of the four proflavine molecules and two sulphate ions in the crystallographic asymmetric unit. Dashed lines represent intramolecular interactions between these; other interactions with neighbouring molecules, and water molecules, have been omitted for reasons of clarity. The right-hand view represents a 90° rotation about the vertical axis, with respect to the left-hand one.



the latter being particularly intimately involved. Three of the four proflavines are strongly hydrogen—(and electrostatically) bonded from their central ring nitrogen atoms, to this sulphate. The fourth molecule is attached by an amino nitrogen, to both sulphate ions. The charged nitrogen to sulphate-oxygen interactions are strong ones, with nitrogen-oxygen distances being between 2.70 and 2.87 Å. Thus, the overall arrangement is dominated by tight hemispherical clusters around the central sulphate ion. The water molecules serve mainly to act as a means of hydrogen-bonding the units together, with involvement of the proflavine amino-nitrogen atoms, as well as the charged sulphate ions. The overall hydrogen-bonding scheme has almost, though not quite, satisfied fully the hydrogen-bonding capability of the various groupings.

A distinctive feature of the crystal structure is the unimportance of ring system stacking. Of the four molecules in the asymmetric unit, only two overlap to any appreciable extent, although all four have their long axis pointing in roughly the same direction. There is no stacking between adjacent molecules in symmetry-related units. This situation contrasts notably that observed in the rather simpler proflavine dichloride crystal structure⁹, as well as in many other aminoacridines¹⁰.

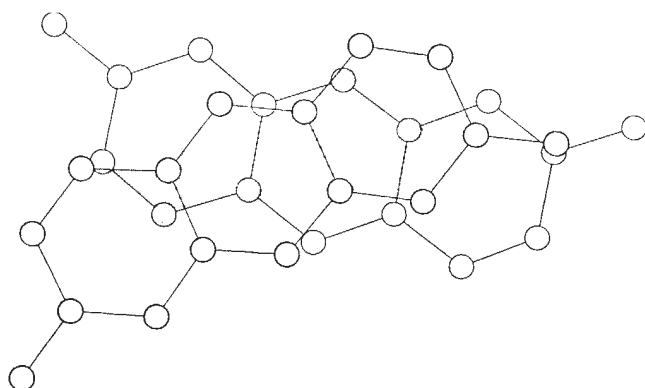


Fig. 3 Skew stacking of two proflavine cations.

These all have straightforward crystal structures with considerable parallel molecular overlap. The unusual skew stacking observed here is shown in Fig. 3. The two ring systems are not related by any symmetry elements, crystallographic or non-crystallographic. One molecule is twisted relative to the other by 150°, and the charged nitrogens are on opposite sides. The two molecules are almost planar, with a separation of 3.34 Å.

It is interesting to note that proflavine dimerisation in solution has been suggested by a number of workers¹¹ to involve stacking. Whether this is similar to that observed here in the solid state, is of course uncertain. Our studies further suggest that close stacking and aggregation may be relatively unimportant in the external binding of proflavine to the phosphates of a DNA helix, certainly compared to any electrostatic interaction.

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Amino acid sequence similarity between cytochrome *f* from a blue-green bacterium and algal chloroplasts

"THE biological gap which separates bacteria and blue-green algae from all other cellular organisms represents one of the largest evolutionary discontinuities in the present day living world"¹. The origin of eukaryotic cells from simpler precursors² is an area of study where speculation is easier than experimental investigation. It seems unlikely that definitive fossil evidence for such early events will ever be obtainable, but molecular methods may be able to give some insight into evolutionary connections between eukaryotes and prokaryotes.

The blue-green algae, unlike other photosynthetic bacteria, carry out oxygenic photosynthesis. The whole photosynthetic apparatus of the blue-green algae bears much more resemblance to that of the chloroplast than it does to that of the other groups of photosynthetic bacteria. The chlorophylls, carotenoids and phycobiliproteins of the blue-green algae are particularly similar to those of the red algae³. Similar electron transport components are found in both blue-green algae and in chloroplasts⁴.

Photosynthetic eukaryotes contain cytochrome *f* (ref. 5), which has a cytochrome *c*-type spectrum and a high redox potential and functions as one of the links between photosystems 1 and 2. In higher plants this cytochrome *f* is membrane bound and is isolated in an aggregated, although homogeneous form. The cytochrome of corresponding function can be readily isolated from many algae⁶ as a small acidic monomeric protein. Comparable cytochromes have been found in blue-green algae^{7,8}. Other types of photosynthetic bacteria contain a wide variety of cytochromes *c*, but none yet studied is very similar to the algal cytochromes *f* either in physical properties⁹ or in amino acid sequence^{10,11}. The cytochrome *c*-555 from green photosynthetic bacteria^{12,13} is the most similar in properties to the algal cytochromes.

Here we have isolated an *f*-type cytochrome from the blue-green alga, *Spirulina maxima*, and determined the amino sequence. We compare this sequence with the known sequences of algal cytochromes *f*, those of the chrysophyte *Monochrysis lutheri*¹⁴, the euglenoid *Euglena gracilis*¹⁵, the phaeophyte *Alaria esculenta* (M. V. Laycock, personal communication), and the rhodophyte *Porphyra tenera* (R.P.A. and R.G.B., unpublished).

The cytochrome *f* was isolated from dried cells of *Spirulina maxima*. The cells were suspended in 0.06 M Tris-HCl, pH 8.0, and broken in a Manton-Gaulin homogeniser. The cytochrome was isolated from the 33-90% saturated ammonium sulphate fraction by chromatography on DEAE-cellulose at pH 8 and by gel filtration through Sephadex G-100. Phycobilins were eliminated with each operation. The cytochrome was finally separated into three fractions by chromatography on DEAE-cellulose (Whatman DE-11), adsorbing the protein from 0.001 M Tris-HCl, pH 8.0, washing the column with 0.01 M buffer, and finally separating and slowly eluting the cytochrome bands with 0.02 M buffer. Each cytochrome was then fractionated with ammonium sulphate and in each case the 60-80%

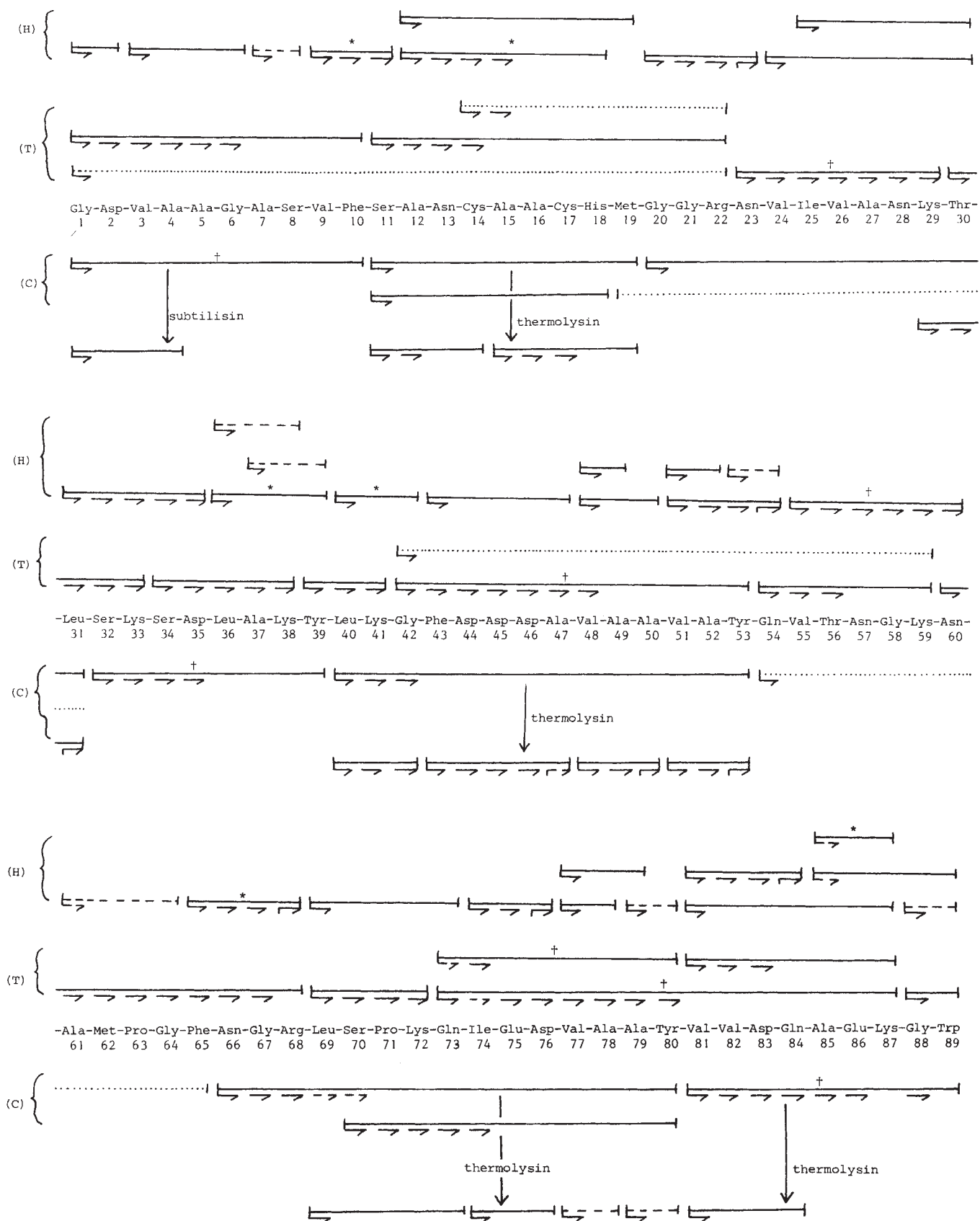


Fig. 1 Amino acid sequence of *Spirulina maxima* cytochrome *f*. Peptides derived by tryptic (T) and thermolysin (H) digestion are shown above the sequence, and by chymotryptic digestion (C) below the sequence. Vertical arrows show peptides produced by further digestion. —, Quantitative amino acid analyses (substandard^{15,16} if marked*); ---, qualitative analyses; . . . , peptides that have definitely been identified as being present in digests but not isolated free from other peptides. — - - - Indicates end groups and subsequent residues revealed by phenyl isothiocyanate degradation, and identified by the dansyl method (substandard^{15,16} if shown - - -); — - - - - , final residues of peptides identified as the free amino acid after the removal of the remainder by phenyl isothiocyanate degradation. † Peptides examined by carboxypeptidase A digestion.

saturated precipitate was collected. During purification, the buffers were kept 0.0001 M in mercaptoethanol or dithiothreitol, which kept the cytochromes in their reduced forms. The final yield of pure protein from 2.8 kg dry cells was about 18 μ mol cytochrome, divided among the three fractions. Portions of each of the three cytochrome fractions which were left standing for four months at 4° C in approximately 70% saturated ammonium sulphate containing 0.02 M Tris-HCl, pH 8.0, formed crystals.

The amino acid sequence was determined by methods similar to those used for some other prokaryotic c-type cytochromes^{15,16}. The haem was removed by treatment with HgCl₂ in 0.1N HCl/8M urea, the apoprotein digested with different proteases, and the peptides fractionated by gel filtration, paper electrophoresis and paper chromatography. Peptides were analysed quantitatively for amino acid composition and to assess purity, and sequences determined by the dansyl phenyl isothiocyanate method, by secondary digestion with other proteases and by investigation with carboxypeptidase A. Amide groups were assigned on the basis of electrophoretic mobilities of small peptides, and by the identification of the C-terminal amino acid of peptides by carboxypeptidase release or after the removal of all other amino acids by phenyl isothiocyanate degradation. The peptides isolated and the sequence deduced are shown in Fig. 1.

The proposed sequence contains two -Asn-Gly- sequences (57/58 and 66/67). Such sequences are well known to be particularly susceptible to deamidation¹⁷, especially at alkaline pH. The yields of peptides derived from these regions from the tryptic and chymotryptic digests were very low, and deamidated peptides were also recognised. For these digests, the apoprotein had been separated from urea and HgCl₂ by gel filtration in 0.1 M ammonia solution, as the apoprotein, in common with several other apocytochromes *f*, was insoluble in dilute acid and the peptides had also been eluted from paper with dilute ammonia. For the thermolysin digest, care was taken to avoid alkaline pH; the apoprotein was separated by gel filtration in 50% (v/v) formic acid, the enzymic digestion was carried out at pH 7.5, and peptides containing the susceptible sequences were eluted from paper with M acetic acid. This gave a good yield of the relevant peptides with electrophoretic mobilities that confirmed that asparagine and not aspartic acid was present. They could be quantitatively deamidated

by treatment with 2 M ammonia solution at 37° C for 5 h.

The three ionically distinct forms of the cytochrome *f* were separated by ion exchange chromatography in relative yields (in order of elution from DEAE-cellulose) of 30%, 60% and 10%, respectively. The forms were not distinguished spectrally or by amino acid analysis, and no differences were seen between the thermolysin peptide maps of the three apoproteins. It had been anticipated that the differences might be due to differential deamidation of the susceptible residues 57 and 66, but the peptide maps did not give any support to this hypothesis. The tryptic and chymotryptic peptides (Fig. 1) were isolated from the predominant form, from digests of 2.4 μ mol and 2.0 μ mol apoprotein respectively. The three forms were independently digested with thermolysin, but when the peptide maps showed no differences, the peptides were pooled for purification, resulting in 4.7 μ mol apoprotein for the combined digest. All the thermolysin peptides, even minor peptides isolated in very low yield, were compatible with the sequence shown in Fig. 1, and so there is at present no evidence as to the cause of the ionic differences between the three forms of the cytochrome *f*.

S. maxima cytochrome *f* has been under investigation in other laboratories. Before the present sequence studies began, a sequenator experiment (R. Holton and J. Ramshaw, personal communication) had identified residues 1-7, 9, 10, 12, 13, 15, 16, 23-25 and 27.

The known cytochrome *f* sequences are shown aligned in Fig. 2. It will be seen that there are several regions of good match, particularly around the haem attachment site (residues 14-17) and around methionine-62, the residue that is likely to function as the sixth iron ligand¹¹. In the middles of the sequences there are regions about twenty residues long (positions 34-52) which are very varied, and which are of slightly different lengths in different cytochromes *f*. Pettigrew¹¹ has discussed possible similarities between (*Euglena*) cytochrome *f* and other types of cytochrome *c* from bacteria and eukaryotes.

The sequences have not been compared by elaborate statistical methods, but a crude similarity matrix (Fig. 3) shows that the blue-green algal cytochrome *f* is at least as similar to each of the eukaryotic cytochromes *f* as the latter are to each other, and is considerably closer to the average sequence than is the *Euglena* protein. The distinctiveness of the *Euglena* sequence is in keeping with the many other

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
a	Gly	Asp	Val	Ala	Ala	Gly	Ala	Ser	Val	Phe	Ser	Ala	Asn	Cys	Ala	Ala	Cys	His	Met	Gly	Gly	Arg	Asn	Val	Ile	Val	Ala	Asn	Lys	Thr	Leu
b	Gly	Asp	Ile	Ala	Asn	Gly	Glu	Gln	Val	Phe	Thr	Gly	Asn	Cys	Ala	Ala	Cys	His	Ser	Val	Glx	Glx	Glx	Mml	Thr	Leu	Glu	Leu	Ser	Ser	Leu
c	Ala	Asp	Leu	Asp	Asn	Gly	Glu	Lys	Val	Phe	Ser	Ala	Asn	Cys	Ala	Ala	Cys	His	Ala	Gly	Gly	Asn	Asn	Ala	Ile	Met	Pro	Asp	Lys	Thr	Leu
d																															
e	Ile	Asp	Ile	Asp	Asn	Gly	Glu	Asp	Ile	Phe	Thr	Ala	Asp	Cys	Ser	Ala	Cys	His	Ala	Gly	Gly	Asn	Asn	Val	Ile	Met	Pro	Glu	Lys	Thr	Leu

	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62
a	-Ser	Lys	Ser	Asp	Leu	Ala	Lys	Tyr	Leu	Lys	Gly	Phe	Asp	Asp	Asp	Ala	Val	Ala	Ala	Val	Ala	Tyr	Gln	Val	Thr	Asn	Gly	Lys	Asn	Ala	Met
b	-Trp	Lys	-----	Ala	Lys	Ser	Tyr	Leu	Ala	Asn	Phe	Asn	Gly	Asp	Glu	Ser	Ala	Ile	Val	-----	Tyr	Gln	Val	Thr	Asn	Gly	Lys	Asn	Ala	Met	
c	-Lys	Lys	-----	Asp	Val	-----	Leu	Glu	Ala	Asn	Ser	Met	Asn	Thr	Ile	Asp	Ala	Ile	Thr	-----	Tyr	Gln	Val	Gln	Asn	Gly	Lys	Asn	Ala	Met	
d	-Ser	Lys	Thr	Ala	Ile	Glu	Glu	Tyr	Leu	Asp	Gly	Gly	Tyr	-----	Thr	Lys	Glu	Ala	Ile	Glu	-----	Tyr	Gln	Val	Arg	Asn	Gly	Lys	Gly	Pro	Met
e	-Lys	Lys	-----	Asp	Ala	-----	Leu	Ala	Asp	Asn	Lys	Met	Val	Ser	Val	Asn	Ala	Ile	Thr	-----	Tyr	Gln	Val	Thr	Asn	Gly	Lys	Asn	Ala	Met	

	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92
a	-Pro	Gly	Phe	Asn	Gly	Arg	Leu	Ser	Pro	Lys	Gln	Ile	Glu	Asp	Val	Ala	Ala	Tyr	Val	Val	Asp	Gln	Ala	Glu	Lys	Gly	Trp			
b	-Pro	Ala	Phe	Gly	Gly	Arg	Leu	Glu	Asp	Asp	Glu	Ile	Ala	Asx	Val	Ala	Ser	Tyr	Val	Leu	Ser	Lys	Ala	Gly						
c	-Pro	Ala	Phe	Gly	Gly	Arg	Leu	Val	Asp	Glu	Asp	Ile	Glu	Asp	Ala	Ala	Asn	Tyr	Val	Leu	Ser	Gln	Ser	Glu	Lys	Gly	Trp			
d	-Pro	Ala	Trp	Glu	Gly	Val	Leu	Ser	Glu	Asp	Glu	Ile	Val	Ala	Val	Thr	Asp	Tyr	Val	Tyr	Thr	Gln	Ala	Gly	Gly	Ala	Trp	Ala	Asn	Val
e	-Pro	Ala	Phe	Gly	Ser	Arg	Leu	Ala	Glu	Thr	Asp	Ile	Glu	Asp	Val	Ala	Asn	Phe	Val	Leu	Thr	Asx	Glx	Asx	Lys	Gly	Trp	Asp		

Fig. 2 Alignment of the amino acid sequences of cytochromes *f* from blue-green bacteria and eukaryotic algae. *a*, *Spirulina maxima* (blue-green bacterium); *b*, *Monochrysis lutheri*¹⁴ (chrysophycean alga); *c*, *Porphyra tenera* (red alga; R. P. A. and R. G. B., unpublished); *d*, *Euglena gracilis*¹¹; *e*, *Alaria esculenta* (brown alga; M. V. Laycock, personal communication). Mml, ε-N methyl lysine. Amides at positions 5, 8 and 13 in sequence, *b*, have been assigned since original publication¹⁴ (M. V. Laycock, personal communication).

properties which divide the euglenoids from other algae¹⁸. The blue-green algal cytochrome *f* does not seem to be appreciably closer to any of the other bacterial cytochromes *c* than are the eukaryotic cytochromes *f*, but no rigorous searches for similarity have been made.

There seem to be three possible explanations for the close sequence similarity between the algal prokaryote and the algal eukaryote cytochromes *f*. First, the whole genome of the eukaryotic cells are evolutionarily derived from a prokaryote closely related to the blue-green alga; or, second, the eukaryotic chloroplasts are derived from a prokaryote related to the blue-green alga, but the remainder of the eukaryotic genomes are derived from different precursors; or, third, transfer of the cytochrome *f* genes (or of a cluster of genes) has taken place in one direction or other between the blue-green algal line and a eukaryote ancestor. The sequence similarity among the cytochromes *f* is so great that convergence cannot be considered a reasonable possibility.

On the present evidence it is not possible to distinguish between these three hypotheses, and it is not easy to design experiments that would rigorously distinguish between them. Much more sequence evidence will obviously be

	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>
<i>a</i>	100	47	53	42	48
<i>b</i>	47	100	48	35	45
<i>c</i>	53	48	100	35	67
<i>d</i>	42	35	35	100	37
<i>e</i>	48	45	67	37	100

Fig. 3 Similarity matrix for amino acid sequences of cytochromes *f* from blue-green bacteria and eukaryotic algae. *a*, *Spirulina maxima*; *b*, *Monochrysis lutheri*; *c*, *Porphyra tenera*; *d*, *Euglena gracilis*; *e*, *Alaria esculenta*. The values shown are matches per 100 residues. For the comparison Asx is taken as being equal to both Asp and Asn, and Glx to both Glu and Gln.

needed, both from cytochromes *f* (particularly from other blue-green algae) and from other proteins and nucleic acids. It is important that parts of the blue-green algal genome concerned with functions other than photosynthesis should be compared with the corresponding parts in eukaryotes, for if the second hypothesis (the endosymbiont theory) were correct, the blue-green algal functions might be expected to have atrophied in the chloroplast. By some criteria, such as DNA base composition^{19,20}, the blue-green algae are a very heterogeneous group, but their photosynthetic mechanisms seem to be very much more uniform^{3,21}. This finding is consistent with the second and third hypotheses. Results from bacteria, particularly on the sporadic distributions of homologous proteins^{10,15,16}, suggest that much gene transfer has taken place. If such events (the third hypothesis) have affected a significant proportion of the genomes of a set of organisms over a period of time, then a phylogeny for the set as whole organisms will not exist. Sequence studies would then not be able to provide results from which a tree relating the major groups of organisms² could be deduced, but may be able to show that such trees are a meaningless oversimplification of what must actually have happened.

Very recently N-terminal sequence homology has been reported between the C-phycoyanins of blue-green bacteria and the corresponding proteins of *Cyanidium caldarium*²², a eukaryotic alga of anomalous properties.

This work was partially supported by National Institutes of Health and National Science Foundation grants to Dr

M. D. Kamen. We thank Drs M. D. Kamen, T. E. Meyer and R. W. Holton for their interest and advice, and Dr M. V. Laycock for permission to include his unpublished *A. esculenta* sequence in Fig. 2. *S. maxima* cells were a gift from Ing. Hubert Durand-Chastel, Sosa Texcoco S.A., Sullivan 51, Mexico 4, D.F.

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Corrigendum

The Editor has been advised that the authorship of the paper "Synthesis mimics of insect juvenile hormone" (*Nature*, **232**, 486; 1971) does not accurately reflect the work done on the paper. With the concurrence of all the present authors, the name of Hwalin Lee (then of Stauffer Chemical Company) should be added as first author.

Errata

In the Matters Arising contribution "Changes in the latitude of the climatic zones of the Northern Hemisphere" by M. K. Miles and C. K. Folland (*Nature*, **252**, 616, 1974) the following corrections are necessary. The second author's name should read Folland not Follard, in the legend to Fig. 2 1970–37 should read 1970–73 and in the legends to Figs. 1 and 2 subpolar flow should read subpolar low.

In the article "Particle acceleration in planetary magnetospheres" by M. J. Houghton (*Nature*, **251**, 205; 1974) equation (1) should read $\sigma_{\parallel} \approx \omega_{pe}/(4\pi \times 10^8)$ mho m⁻¹ and not as printed.

In the article "Acetylcholine as an excitatory neuromuscular transmitter in the stomatogastric system of the lobster" by E. Marder (*Nature*, **251**, 730; 1974) choline acetyltransferase was printed incorrectly as acetylcholine transferase on three occasions. These were in the heading to Table 1, in the first line of the penultimate paragraph on page 730 and in the second line of the last paragraph of the article.

reviews

Independence and Deterrence: Britain and Atomic Energy, 1945–1952. By Margaret Gowing. Vol. 1: *Policy Making*; pp. xi+503. Vol. 2: *Policy Execution*; pp. xiv+559. (Macmillan: London and Basingstoke, November 1974.) £10.00 each.

THESE two volumes of official history are notable for Professor Gowing's mastery of her subject, the orderliness of her presentation of a complex story and the clarity of her style. She has much to say which scientists will find absorbing, and for the non-scientist she manages to explain as much of the technology as he is likely to be able to assimilate. Even more valuably she reveals the intimate processes of government with a wealth of the sort of detail which the public is rarely allowed to know until at least 30 years have elapsed. The earlier release in this instance indicates how quickly this initial phase of atomic policy has passed into history. "Very little", we are told, "has been modified or omitted on public interest grounds".

Some early comments about the book, because they focused on Professor Gowing's more critical passages, gave the impression that her work was a revelation of governmental confusion and muddle, with parliament and the nation being kept in the dark while objectives were being pursued which had never been adequately discussed, even within the government or in Whitehall. The criticisms are there all right but the overall impression derived from reading the two volumes is different. The major decision which the new government took after 1945—to develop a national atomic programme, with a national atomic bomb as its top priority—did indeed emerge in a somewhat messy way. Most of the scientists and officials engaged in the preliminary arrangements seem to have acted on the assumption that they were intended

to produce atomic weapons, well before the ministerial decision was finally taken early in 1947. Parliament, meantime, had shown little curiosity and when it was formally told in May 1948 that atomic weapons were being developed there was scarcely a ripple on the political waters.

Once that decision had been taken, the necessary priority was given to the project and was subsequently upheld by Prime Minister Attlee whenever it needed to be re-asserted.

The result was the trial explosion of the bomb at Monte Bello in 1952, which was a major technical achievement. The only possible criticism of the execution of the policy—that it might have been completed sooner—is shown by Professor Gowing to be of little importance. Given the overall limitation of British resources in manpower, money and materials, any speeding up could have amounted to no more than

a few months. Indeed, in both timing and cost the operation came much closer to the original estimate made for it than most other projects involving frontier technology have been able to claim. It was undeniably a success story.

The only inside critics of any weight who questioned the decision to make the bomb, were Patrick Blackett and Sir Henry Tizard. Blackett's memorandum of 1947, reproduced in full in these books, was a clear statement of a particular point of view, and no doubt it deserved fuller discussion than it received. But its rejection is not surprising. For Blackett made assumptions about the likely attitudes of the United States and the Soviet Union and also about the likely effect upon other countries of Britain deciding not to make atomic weapons, which were shared by scarcely any politicians or civil servants at the time and by only

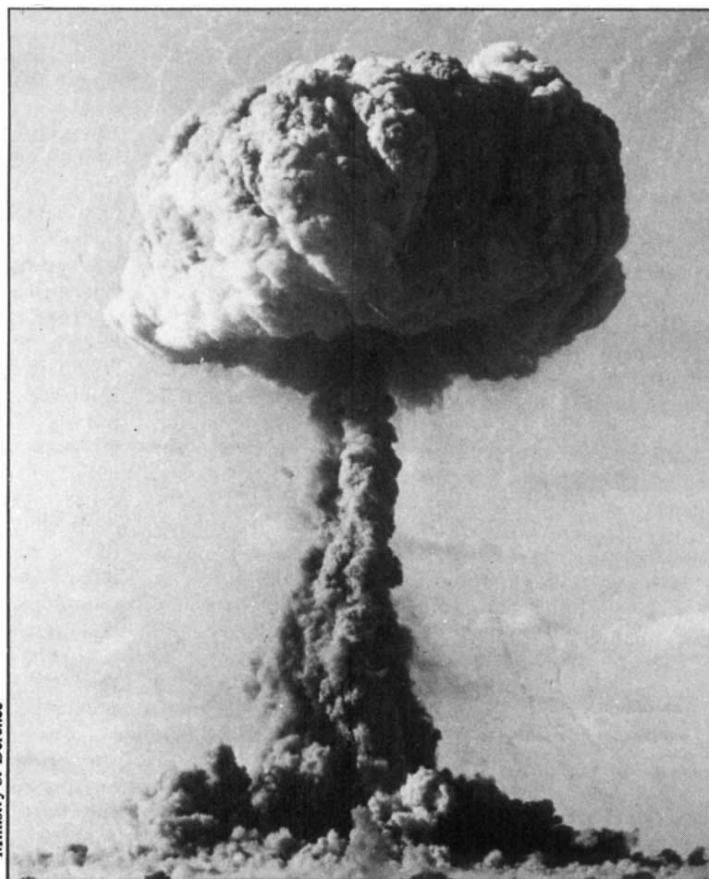
a few of his fellow scientists. Even in retrospect his forecasts of what might result if various alternative policies were followed seem little closer to what actually occurred than do the predictions of anyone else.

In 1949 Sir Henry Tizard, having perceived sooner than most that Britain's relegation from the ranks of the great powers was likely to be irreversible, argued that she could not afford this symbol of great power status, which she would in any case be unable to make effective. But it seems that he would have wished to divert the funds into other defence programmes rather than into civilian atomic projects, about which he was unduly pessimistic.

Despite Professor Gowing's admirably balanced narrative, it is not always easy for the reader to keep in mind the developing international scene which provided the background for atomic policy. At the beginning, the future attitudes of all the wartime allies were in doubt; not the least of

The way it happened

Kenneth Younger



Ministry of Defence

these doubts concerned the likely attitudes of the US and USSR to one another. By the end of 1947 the Council of Foreign Ministers had finally broken down and anxiety about Soviet Policy in Europe was growing, reaching a climax with the Berlin blockade in the following year. British politicians, who had only just emerged from the second world war, found the outbreak of a third entirely credible. Moreover, they remembered just how it had felt in the pre-war years to be militarily outclassed by a potential enemy; and the only way to avoid a repetition of that was to cling to the American alliance. They were, however, still unsure, even in 1949, of permanent American military commitment in Europe and therefore found it impossible to contemplate throwing away the one major weapon in the development of which, they had reason to believe (though wrongly as it turned out), they were ahead of the USSR.

As regards the implementation of the programme, the heroes of the story are unquestionably the three men who led the main institutions entrusted with research, production of fissile material and weapons: John Cockcroft, Christopher Hinton and William Penney. All were supremely competent in their respective specialities and all showed an exceptional capacity for leadership of their teams. What stands out from Professor Gowing's story is that the quality of these men and their capacity to cooperate with one another triumphed over the complicated bureaucratic framework within which they had to operate. Even the absence, at certain periods, of clear central direction and coordination may have had its compensations in the flexibility which it allowed the scientists in changing their methods according to the state of the art. The supervising department—the Ministry of Supply—also deserves credit for having been capable of adjusting many civil service practices to the needs of a wholly unprecedented pioneering operation. In contrast, industry, with a few honourable exceptions, proved unimaginative and inadequate.

At the point when these volumes break off in 1952 a new phase, on which we are promised further volumes, was opening. Slowly the implications of the new weaponry—moral, political and military—came under public discussion. Almost equally slowly a realisation of Britain's changed world status came home to public opinion and with it an altered appreciation of the comparative importance of Britain's relations with the United States (which dominates the volumes already published), the Commonwealth and Europe.

How far the policies followed from

1945–52 may have prejudiced the subsequent evolution of British atomic policy, for better or for worse, will no doubt emerge as the story unfolds. 'Very little' would be my present verdict. What was done, I believe, in the immediate post-war years had to be done in the confused circumstances of that time, and in that sense was correct. The next phase was entirely different. Events such as the end of the Stalin era, the coming of the hydrogen weapon and the space programmes, and the rapid reduction of Britain's responsibilities as a world power from the late 1950s onwards combined to require a reassessment of Britain's role in nuclear weaponry.

Whether the choices which Britain then made were wise or not, we shall

hope to learn from later volumes, but I do not think that they were prejudged by what had gone before, except in the sense that if Britain had had no atomic programme she would have had little or no choice to make. For myself, having spent the years 1945–52 as a Labour MP, with a spell in the Foreign Office as a Minister (1950–51), I am a little surprised to find nothing in these volumes which, had I known of it at the time, would have radically altered my view of the role Britain should play. Nor would I have dissented from the atomic programme which was under way.

If this sounds a little like saying that because it happened this way, this was the way it had to happen, I can't help that. □

Varying aspects of fisheries work

Sea Fisheries Research. Edited by F. R. Harden Jones. Pp. xvii+510. (Elek Scientific Books: London, March 1974.) £10.00.

THIS book is a collection of 21 essays in honour of Michael Graham, former Director of Fishery Research with the Ministry of Agriculture, Fisheries and Food at Lowestoft. Graham commenced his career in fisheries research in 1920, and his early work on the North Sea cod and its fishery was, and still is, a model of investigation into fish biology, life cycles, spawning grounds, and age compositions. The study led to proposals for the better management of the fishery and was an outstanding contribution to the so-called theory of fishing, whereby attempts are made to establish parameters for growth, recruitment, and natural and fishery mortality. After the 1939–45 war Graham returned to Lowestoft as Director of Fishery Research, and immediately began planning for the postwar research effort by building vessels and recruiting staff of a calibre which has made the Fisheries Laboratory at Lowestoft, and its satellites at Conwy and Burnham-on-Crouch, respected throughout the world. Graham retired in 1958 and characteristically applied himself to the environmental reclamation of colliery waste tips, and to lecturing. Sadly, he did not live to see the publication of this book, but one feels that he would have approved of this collection of essays by his former colleagues.

Naturally, the varying aspects of fisheries work are well covered. Papers on the exploitation of stocks of fish and shellfish are much in evidence. M. J. Holden's thoughtful paper on the rational exploitation of elasmobranch populations suggests how vulnerable to extinction most of them would become

if heavily exploited. That is supported by A. C. Burd's requiem for the North East Atlantic herring and its virtually extinct fishery, a classical example of the failure of some fishery scientists to accept the evidence of others, and the consequent lack of international co-operation. The world resources of hakes of the genus *Merluccius* are discussed by B. W. Jones, who suggests that several species are still underexploited, although some, notably the European hake (*M. merluccius*), have been heavily overfished. In an essay on the world's industrial fisheries (that is, fisheries which produce fish meal and oil), C. T. Macer confirms that some hakes, especially the western South American species-group (*M. gayi*), might support a greater fishery than at present. A contribution from P. R. Walne discusses the problems of the shellfish industries of England and Wales. He also considers the fascinating possibilities allowed by the development of successful culture techniques, for which most of the biological requirements have already been established.

It is suspected that the pollution of inshore waters is one of the factors responsible for the low rate of recruitment of young individuals to many native shellfish populations. In the Essex oyster fishery the total catch declined as the human population, and the consequent pollution of the area, increased. H. A. Cole takes a broader look at pollution in his essay on marine pollution and the UK fisheries, and he concludes that there is little evidence that the discharge of industrial and domestic waste into the sea has had a deleterious effect on sea fisheries. In support of this he cites the increase in landings of plaice, cod, and haddock in the North Sea, and plaice and cod in the Irish Sea (both are enclosed and relatively polluted seas). Changes of

this nature may be, however, a direct result of fluctuations in year-class strength, and of changing climatic conditions; both factors may mask the adverse effects of pollution.

Several of the essays are concerned with technical subjects. R. W. Blacker's discussion of recent advances in otolith studies is an important contribution to the methods of study and interpretation of otoliths for age determination (a 'traditional' fisheries' tool). By contrast, Alan Jamieson and C. E. Purdom present new techniques in fisheries research with essays on the study of genetic tags for marine fish stocks, and genetic variation in fish. The volume also contains a contribution on the design of research vessels by Geoffrey C. Trout, as important a study as any attempted at Lowestoft (and as one who has sailed on FRV *Cirolana*—the newest of the fleet of research vessels—I can vouch for the excellence of the product).

The quality of production of this book is excellent, although the photographic illustrations do not reproduce well. The editing is even and unobtrusive and must have represented a formidable task for the editor. Altogether this is an admirable and fitting volume in honour of Michael Graham.

Alwyne Wheeler

Liquid crystals

The Physics of Liquid Crystals. By P. G. de Gennes. (The International Series of Monographs on Physics.) Pp. xi+333. (Clarendon: Oxford; Oxford University Press: London, June 1974.) £11.50.

THIS is the first modern book to survey and discuss the physical properties of liquid crystals. Liquid crystals combine some of the properties of the crystalline and of the isotropic liquid phases; they have received considerable attention during the last 10 years. The re-discovery of electro-optical effects, which are used in liquid crystal display devices, has led to a revival of basic research on the physical and chemical properties of liquid crystals, both theoretically and experimentally. Their study is complicated because it involves various physical and chemical disciplines, making it almost impossible to cover all the aspects in one book. Although the main physical topics are discussed or at least mentioned in this book, the main emphasis is on hydrostatic and hydrodynamic properties which, in principle, also determine the optical properties.

In Chapter 1 there is a short introduction to the nature of the different liquid crystalline phases and of the constituent molecules. The following four

chapters, the major part of the book, deal with the nematic liquid crystalline phase which is characterised by long range, orientational ordering of the constituent, rod-like molecules.

Chapter 2 deals with long and short range order. Different order parameters are introduced and linked to measurable quantities, such as nuclear magnetic resonance spectra, the anisotropy of magnetic susceptibility, and so on. Some theories of orientational ordering are reviewed briefly. Finally, short range order effects near the transition from the isotropic to the nematic phase are discussed. The static, elastic distortions in a nematic single liquid crystal are treated in Chapter 3. There, a hydrostatic theory based on a continuum description is introduced and discussed thoroughly, and the role of boundary effects and boundary conditions is made clear. Various applications of the theory, and some instructive problems are given. Most are concerned with deformations induced by external magnetic and electric fields for different boundary conditions. Close attention is also given to the strong natural light scattering, which is analysed in terms of spontaneous orientation fluctuations.

Chapter 4 describes distortions that are not continuous but which involve singular points or lines. These defects, where the orientation varies discontinuously, are called disclinations. In connection with disclinations different textures are discussed.

An understanding of the coupling between orientation and flow is essential to any consideration of hydrodynamic properties. That problem, and the different approaches to it, are discussed thoroughly in Chapter 5. Again various applications and experimental situations are described, including classical flow experiments, flow induced by external fields, inelastic light scattering, dynamic scattering, and so on.

The cholesteric liquid crystalline phase, essentially a helically distorted nematic phase, is also discussed. Because of the helical structure this phase has rather peculiar optical properties which are discussed in a rather formal way. The hydrostatic and hydrodynamic theories of the nematic phase are extended to a discussion of the cholesteric phase. The static distortion of the helical structure by external fields, flow properties, the analogue of dynamical scattering and other typical phenomena are considered and some typical defects and textures are also described.

The last chapter is devoted to smectic liquid crystals. In addition to the orientational ordering they have a layer structure. First, a classification and description of the different smectic phases is given, and then attention is restricted

to the A and C-phases. In the discussion of the static and dynamic properties the role of the layer structure is clearly displayed. Again, attention is given to distortions induced by external fields and mechanical forces, to light scattering, the propagation of acoustic waves and flow properties. The chapter ends with a description of phase transitions and precritical phenomena.

Though various experimental methods and experiments are mentioned or briefly described, the main emphasis is on the theoretical aspects. The approach to these aspects, which is purely phenomenological, is based on the fundamental hydrostatic and hydrodynamic equations. The study of chapters 3 and 5, which form the central part of the book, is therefore necessary before the chapters on the cholesteric and smectic phases can be understood. One may regret that little or no attention has been given to molecular theories and considerations, as these have been quite successful and instructive. Some of the discussions are rather formal and general, whereas others are more concise, but the material is nevertheless presented in a clear and systematic way.

This is a most valuable book which gives a comprehensive though not easy introduction to many aspects of the liquid crystalline phases.

W. J. A. Goossens

NEW FROM DENT

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Edited by R. H. STOY
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Energy and fat

Energy Balance and Obesity in Man.
By J. S. Garrow. Pp. xii+335. (North
Holland: Amsterdam, London; American
Elsevier: New York, 1974.) Dfl.80;
\$30.80.

THIS is a very well written, highly persuasive, important book which provides all the basic and practical information necessary for an understanding of the problem of obesity.

The trouble with 'obesity' is that it produces emotional reactions which people rationalise from a literature that provides any evidence desired. In medical science there can be few pursuits which affect such large numbers of people with such little effect, as the investigation and treatment of obesity. Of course, few things are as satisfying to the intellectual academic as a problem with little prospect of application to a real-life situation, and that may explain the large volume of contradictory literature on this subject.

In a slightly lengthy introduction, Dr Garrow initiates us into his positive interpretation of this mass of confusing information. His book reviews comprehensively the different methods used to measure the intake and expenditure of energy in man, the theories of the control of energy intake, the part played by physical activity in energy expenditure, and the variable composition of the human body in relation to fat and how this can be measured. The final section is concerned with the difficult, practical problems of how to diagnose and classify an obese patient and includes a critique of the likely effectiveness of the various forms of treatment.

My only serious criticism of this excellent book is that, possibly because the author has apparently become actively interested in obesity only comparatively recently, his interpretation frequently seems biased and somewhat selective. He fails to quote certain relevant literature, especially from the large volume of physiological papers on exercise, and he is perhaps slightly too dogmatic in many of his statements. In those sections in which he has first-hand knowledge—for example, the chapter on "Energy stores: their composition, measurement and control"—he writes clearly and dispassionately in a most stimulating fashion.

I have no hesitation in recommending this book. To my knowledge, it is by far the best book on the subject of energy balance in man. It should be read by everyone involved in the study of obesity—though all should retain certain reserves about accepting totally some of Dr Garrow's views.

J. V. G. A. Durnin

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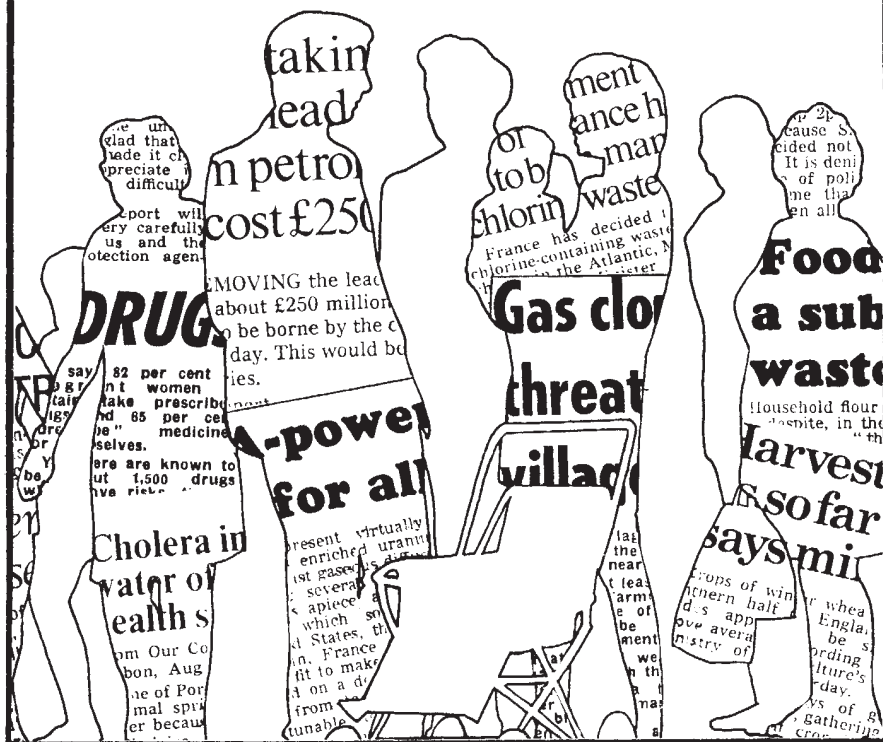
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obituary

S. Petterssen

SVERRE PETTERSSSEN, the outstanding meteorologist, died in London on December 31. He was 76.

Born in Norway, Petterssen served in the Norwegian Meteorological Service from 1924 to 1939 after graduating from Oslo University. In 1939 he became Professor of Meteorology at the Massachusetts Institute of Technology, and came to England in 1942. During the war he was stationed at the Meteorological Office at Dunstable, establishing now standard techniques of upper air analysis. In recognition for his services, he was made CBE in 1948 and Commander of the Order of St Olaf in 1949.

After the war Petterssen returned to Norway, serving in the Norwegian Weather Forecasting Service until 1948, when he was appointed Director of Scientific Services of the US Air Force Weather Service. In 1952, he returned

to academic life as Professor of Meteorology at the University of Chicago. In the next ten years, he played a large part in the rapid expansion of research and education within government and academic institutions.

Petterssen's contributions to the theory of frontogenesis and convection, to the kinematics of weather systems and to the physics of fog were highly original, and led the way towards more quantitative methods of weather forecasting.

H. Heller

HANS HELLER, the distinguished endocrinologist, died on December 30 at the age of 70.

Born in Brno, Professor Heller studied Natural Sciences at Cambridge from 1929 to 1931, when he was

appointed to a post in pharmacology at the University of Vienna. He returned to England in 1935 to obtain medical qualifications at University College Hospital. He worked there as a Beit Fellow until 1941, when he took an appointment at Bristol University, becoming Professor of Pharmacology in 1949.

Heller was particularly famous for his investigations of the water balance effect of neurohypophyseal hormones in amphibia, showing that the water-balance principle differed from that of mammals, later isolating the hormones from cod in 1959. His research into the nature of such hormones in other animals illustrated a pattern for the evolutionary development of the hormones.

From 1963 to 1974, he was editor of the *Journal of Endocrinology* and at the time of his death was chairman of the Society for Endocrinology.

announcements

Awards

New Year's Honours

Max Ferdinand Perutz has been made a Companion of Honour for services to molecular biology.

Knights Bachelor include: **Arthur Llewellyn Armitage**, vice-chancellor, Victoria University of Manchester; **Frederick Arthur Bishop**, Director-General, National Trust; **Harold Montague Finnieston**, FRS, chairman, British Steel Corporation; **Hugh Ford**, FRS, Professor of Mechanical Engineering, Imperial College of Science and Technology; **Ieuan Maddock**, FRS, chief scientist, Department of Industry; **Edward Eric Pochin**, lately director, MRC Department of Clinical Research.

Erik Erikson, Professor of Psychiatry at Harvard University, has been awarded the **Mental Health Association Research Achievement Award** at the organisation's annual meeting in Washington.

Godfrey Hounsfield has been awarded the **Wilhelm Exner Medal**, a major Austrian award, for the invention of the EMI computerised X-ray brain scanner.

Appointments

Henry Francis Black has been appointed to a personal chair of mechanical engineering at Heriot-Watt University.

Miscellaneous

Mitchell Prize. The Mitchell Energy & Development Corporation, in association with the Club of Rome, is to make biennial awards for papers submitted on the problems inherent in the transition from growth to equilibrium of population, material consumption and energy use over the next 40 years. Application deadline for 1975 is February 28. For information contact: Limits to Growth '75, 5645 South Woodlawn Avenue, Chicago, Illinois 60637.

The Food and Drug Administration's OTC Panel is interested in receiving any unpublished data on foetal damage or loss associated with the maternal use of vaginal contraceptives or douches. For information, contact Elizabeth B. Connell, Food and Drug Administration Bureau of Drugs, Division of OTC Drug Evaluation (HFD-510), 5600

Fishers Lane, Rockville, Maryland 20852.

The Moscows Seminar on Collective Phenomena will be held every Sunday at noon, on a broad range of scientific topics. For information, contact I. and V. Brailovsky, pr. Vernadskogo 99, korp(bldg) No. 1, kv (Apt.) 128, Moscow.

Lady Tata Memorial Trust. Applications are invited for scholarships and fellowships to aid research on leukaemia and allied conditions. Further information may be obtained from: Secretary (European) Scientific Advisory Committee, Lady Tata Memorial Trust, Chester Beatty Research Institute, Fulham Road, London SW3.

A. E. Bennett Research Awards. The Society of Biological Psychiatry is offering two awards—one in basic and one in clinical science—for the purpose of stimulating worldwide research in biological psychiatry by young (under 35 years) investigators. Further information from: Chairman, Committee on Research Awards, Veterans' Administration Hospital, Brockton, Massachusetts 02401.

International meetings

March 13–14, **Chromosomal Proteins and Their Role in Regulation of Gene Expression**, Florida (Florida Colloquium on Molecular Biology, Department of Biochemistry, University of Florida, Gainesville, Florida 32610).

April 3–6, **Electrochemistry Symposium**, London (Hon. Secretary, Felix Gutmann, Department of Physical Chemistry, The University of Sydney, NSW 2006, Australia or Dr D. Inman, Imperial College, London, UK).

April 7, **Metal Compounds in Biological Systems**, Sheffield (Dr E. D. McKenzie, Department of Chemistry, University of Sheffield or Dr G. J. Leigh, A.R.C. Unit of Nitrogen Fixation, University of Sussex, UK).

April 7–9, **Food from Waste**, Weybridge (The Secretary, National College of Food Technology, Weybridge, Surrey, UK).

April 7–9, **Interatomic Forces in Condensed Matter**, Reading (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX, U.K.).

April 7–14, **Recycling and Disposal of Solid Waste**, Dubrovnik (Mr R. V. Arnfield, Director, Industrial and Business Liaison Office, University of Nottingham, University Park, Nottingham, NG7 2RD, UK).

April 8–10, **Thermal Regime of Glaciers and Ice Sheets**, Vancouver (R. B. Sagar, Department of Geography, Simon Fraser University, Burnaby, B.C. V5A 1S6, Canada).

April 8–11, **Environmental Biochemistry**, Burlington (Dr Jerome O. Nriagu, Department of the Environment, Canada Centre for Inland Waters, Burlington, Ontario L7R 4A6, Canada).

April 8–11, **Organ Cultures in Biomedical Research**, Norwich (Dr B. M. Richards, Searle Research Laboratories, Lane End Road, High Wycombe, Bucks, HP12 4HL, UK).

April 14–17, **Nuclear Quadrupole Resonance Spectroscopy**, Tampa, Florida (3rd International Symposium on Nuclear Quadrupole resonance, Department of Physics, University of Florida, Gainesville, Florida 32611).

Reports and Publications

Great Britain

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 269, No. 897: Implantation, Foetal Membranes and Early Placentation of the African Elephant *Loxodonta africana*. By J. S. Perry. Pp. 109–136 + 1–7. (London: The Royal Society, 1974.) [111]

British Nutrition Foundation. Annual Report, 1973/1974. Pp. 26. (London: British Nutrition Foundation, 93 Albert Embankment, SE1, 1974.) [111]

Rearing the Hymenoptera Parasitica. By K. G. V. Smith. Pp. 15. (AES Leaflet No. 35.) (London: Amateur Entomologists' Society, 137 Gleneldon Road, SW16, 1974.) 40p. [111]

Index of Current Government and Government-Supported Research in Environmental Pollution in Great Britain, 1973. Pp. 206. (London: Department of the Environment, 2 Marsh Street, SW1P 3EB, 1974.) gratis. [111]

The Universities and Applied Research: Their Relevance to Social and Industrial Needs. (Proceedings of the 1974 Symposium held on 23rd April at the Royal Society, London.) Pp. 90. (London: The Research and Development Society, 47 Belgrave Square, SW1X 8QX, 1974.) £2.50 members; £4.50 non-members. [111]

Report from the Select Committee on Science and Technology—Offshore Technology. (Session 1974.) Pp. 53. (London: HMSO, 1974.) 51p net. [121]

Dinosaurs. By W. E. Swinton. Fifth edition. Pp. vii + 47 (9 plates). (London: British Museum (Natural History), 1974.) 50p. [121]

Another Sword for St. James. By Professor Derek W. Lomax. (Inaugural Lecture delivered in the University of Birmingham on 19th February 1974.) Pp. 19. (Birmingham: The University, 1974.) 25p. [121]

Unconventional Protein Sources: A Guide to Selected Literature and Sources of Information. Pp. 8. Automobile Fuels—The Alternative to Petroleum: a Guide to Selected Literature. Pp. 10. Salmon and Trout: a Guide to Selected Literature and Sources of Information. Pp. 11. (Guidelines.) (London: Publications, Science Reference Library (Bayswater Branch), 10 Porchester Gardens, 1974.) gratis. [131]

Computer Board for Universities and Research Councils. Report of the Computer Board for the period 1st April 1972–31st March 1974. (Cmd. 5775.) Pp. 25. (London: HMSO, 1974.) 26p net. [141]

Institute for Marine Environmental Research. Report 1973/1974. Pp. 71. (Plymouth: Institute for Marine Environmental Research, 1974.) [141]

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Department of the Environment. Annual List of Publications 1973. Pp. vi + 121. (London: Department of the Environment, 1974.) [151]

Royal Observatory Annals. No. 8: Photoheliographic Results 1965. Pp. 42. (Hertsmoeneux: Royal Greenwich Observatory, 1973.) 90p net. [151]

1974/1975 ICI Information Handbook. Pp. 48. (London: ICI, Ltd., 1974.) [181]

Pharmaceutical Industry Press Directory. Pp. 23. (London: The Association of the British Pharmaceutical Industry, 1974.) [181]

The Scientific Proceedings of the Royal Dublin Society. Series A. Vol. 5, No. 11: Poaceae—Irish Members. Part 5: Anatomy. By M. A. Farragher. Pp. 159–198 + plates 17–28. £1.50. Vol. 5, No. 12: Geochemical Evidence for the Original Nature of the Rossare Complex. S. E. Eire. By R. S. Thorpe. Pp. 199–206. 40p. Vol. 5, No. 13: The Glaciations of the Dingle Peninsula, County Kerry. By Colin A. Lewis. Pp. 207–236. £1. Vol. 5, No. 14: The Evolution of North Bull Island, Dublin Bay. By Colin R. Harris. Pp. 237–252 + plates 29 and 30. 50p. Vol. 5, No. 15: Glacial Retreat of Late Midlandian Ice in the Slieve Bernagh. By T. F. Finch. Pp. 253–264. 60p. Series B. Vol. 3, No. 19: A Laboratory Culture Medium for *Rhizobium* from *Lotus pedunculatus*. By M. B. Walsh and P. L. Curran. Pp. 267–272 + plate 12. 20p. (Dublin: Royal Dublin Society, 1974.) [181]

The Zoological Record. 1971, Vol. 108, Section 18: Aves. Compiled by the Staff of the Zoological Society of London. Pp. 264. £11. 1971, Vol. 108, Section 19: Mammalia. Compiled by the Staff of the Zoological Society of London. Pp. 405. £12.50. (London: The Zoological Society of London, 1974.) [181]

University of Nottingham. Report of the School of Agriculture, 1973/1974. Pp. 170. (Sutton Bonington, Loughborough: University of Nottingham, School of Agriculture, 1974.) £1. [201]

The National Institute of Agricultural Botany. Report and Accounts, 1973. Pp. 89. (Cambridge: The National Institute of Agricultural Botany, 1974.) [201]

The Radiochemical Centre. Technical Bulletin 74/3: Mossbauer Sources. Pp. 20. (Amersham: The Radiochemical Centre, 1974.) [211]

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National Institute of Agricultural Engineering. Annual Report, 1st April 1973–31st March 1974. Pp. 80. (Silsoe, Bedford and Penicuik, Midlothian: National Institute of Agricultural Engineering, 1974.) £2. [221]

Greater London Council Scientific Branch. Annual Report of the Scientific Adviser 1973. By R. T. Kelly. Pp. 140. (London: Greater London Council, 1974.) £4.25. [221]

Elements of Organometallic Chemistry. By F. R. Hartley. (Chemical Society Monographs for Teachers, No. 26.) Pp. vi + 103. (London: The Chemical Society, 1974.) £1.20. [251]

Other countries

National Research Council of Canada. Report of the President, 1973/1974. Pp. 133. (Ottawa: National Research Council of Canada, 1974.) [41]

CERN—European Organization for Nuclear Research. CERN 74–19: Supermix—a Multi-Host Front End Concentrator System for Asynchronous Consoles. By T. Bruins, K. S. Olsson, E. M. Pafandri, B. Segal, H. J. Slettenhaar and H. Strack-Zimmermann. Pp. vii + 85. (Geneva: CERN, 1974.) [41]

Neurophysiology of Enlightenment: Scientific Research on Transcendental Meditation. Presented by Dr Robert Keith Wallace. Pp. 48. (Seelisberg, Switzerland: Maharishi International University, 1974.) [41]

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Memoir 378: Geology of Bathurst Island Group and Byam Martin Island, Arctic Canada. (Operation Bathurst Island). By J. Wm. Kerr. Pp. 152 (18 plates). \$5. Paper 73–38: Research in Geochemical Prospecting Methods for Fluorite Deposits, Madoc Area, Ontario. By J. P. Lalonde. Pp. 56. \$3. Paper 73–40: Late-Wisconsin Glaciation of Southwestern Newfoundland (with Special Reference to the Stephenville Map Area). By I. A. Brookes. Pp. 31. \$3. (Ottawa: Information Canada, 1974.) [41]

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